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I II III IV

$$R\text{-}AA_1\text{--}AA_2\text{--}AA_3\text{--}AA_4\text{--}AA_5\text{-}R_1 \qquad (\alpha)$$

(57) Abstract

Pseudopeptides of formula (α) wherein AA_1 is an arginyl residue; AA_2 is a basic amino acid residue, a neutral/nonaromatic amino acid residue or proline residúe or is an N-alkylated (1-6C) form thereof; AA_3 is an aspartic acid or glutamic acid wherein the remaining carboxyl group may be esterified with alkyl (1-6C), or an alanine residue; AA_4 is a neutral/nonaromatic amino acid residue; AA_5 is a neutral/aromatic amino acid residue wherein one or more hydrogens of its aromatic portion can be substituted by NO_2 or halogen, or is a neutral/nonpolar/large/nonaromatic amino acid residue or the N-alkylated (1-6C) form thereof; R is acyl (1-6C), arylsulfonyl, alkylsulfonyl, arylalkylsulfonyl or alkoxycarbonyl group; and R_1 is -OH, $-NR_2R_3$ or $-OR_4$ wherein each of R_2 and R_3 is hydrogen or an alkyl group (1-6C) and R_4 is alkyl group (1-6C); and wherein at least one of the linkages I-IV is a modified peptide linkage $-COCH_2-$, $-CH(OH)CH_2-$ or $-CH_2NH-$, and the remaining linkages are -CONH- or $-CON(CH_3)-$.

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DESCRIPTION

PSEUDOPENTAPEPTIDES WITH IMMUNOMODULATING ACTIVITY

Technical Field

The invention relates to synthetic pseudopentapeptides having immunomodulating activity, to pharmaceutical compositions and to their use in the treatment of immune diseases such as arthritis. More particularly, it concerns improved pseudopentapeptides which are designed to exhibit enhanced stability in serum.

Background Art

The isolation of a 49 amino acid peptide from thymus tissue which was named "thymopoietin" was reported in 1975. (Thymopentin as a generic drug is often referred to as "thypentin." The terms are used interchangeably herein.) In vitro, thymopoietin enhances differentiation of prothymocytes to thymocytes, as well as late stage B-cell differentiation, induction of complement receptor, and lymphoid cell transcription. Its effects in vivo include induction of prothymocytes, T-cell dependent antibody response enhancement, and delays in the onset of autoimmune hemolytic anemia in mice.

By 1979, it had been shown that a synthetic pentapeptide which represents amino acids 32-36 of thymopoietin had the biological activity of the

full-length peptide. Goldstein, G., et al., Science (1979) 204:1309-1310, demonstrated that the penta-peptide Arg-Lys-Asp-Val-Tyr showed these biological effects. Further work as to the activity of the pentapeptide in vivo, including its immunokinetics was reported by Di Perri, T., et al., J Immuno Pharmacol (1980) 2:567-572.

While early work on the thymopoietin hormone and the corresponding pentapeptide thymopentin focused on immunomodulation activity, it was observed in the clinic that these materials had a positive effect with respect to rheumatoid arthritis. See, for example, Malaise, M.G., et al., Lancet (1985) 832-836. While the results of treatment with this pentapeptide are extremely encouraging, it has a major drawback in that its half-life in plasma is only 30 seconds. Therefore, continuous infusion over at least 10-minute periods is required and repeated administration is necessary. But the treatments are successful; the patients in the Malaise et al. study insisted on continuation of treatment, even though it required that they report for continuous infusion treatments a few days each week.

Similar results were obtained by Pipino, F., et al., Arzneim-Forsch Drug Res (1988) 38:116-119. Thus, it appears that the pentapeptide is a simple, as well as nontoxic, valuable drug which would find more convenient and widespread use if it could be stabilized sufficiently

to permit administration in some form other than continuous infusion.

Studies have also been made with regard to the structural requirements for the pentapeptide, and as to whether variations in the amino acid sequence can be made and still result in peptides with the required activity. The earliest such study of which applicants are aware is that of Heavner, G.A., et al., Arch Biochem Biophys (1985) 242:248-255, which assessed the activity of 29 analogs by their ability to induce intracellular cGMP elevation in human T-cells, and their ability to compete with radiolabeled thymopoietin for its T-cell receptor. The results indicated that the arginine residue in position 1 was indispensable for activity, although slight activity could be obtained when it was replaced by the D-isomer. The same was true for the aspartic acid residue at position 3, although again reduced, but not zero activity could be obtained by replacement with the D-isomer.

Specifically, replacing the aspartic acid residue in the 3-position with a glutamyl residue also eliminated both receptor competition activity and cGMP stimulation. This is of interest because thysplenin, a 49 amino acid peptide similar to thymopoietin (differing only in position 34 by replacement of the aspartic acid residue with glutamic acid), was isolated from bovine spleen. A corresponding pentapeptide, splenin, having the sequence

Arg-Lys-Glu-Val-Tyr, shows similar abilities to induce T- and B-cell precursors although their biological activities differ in other respects (Audhya, T., et al., Proc Natl Acad Sci USA (1984) 81:2847-2849. To applicants' knowledge, there have been no clinical trials of splenin with respect to its effect on rheumatoid arthritis.

The Heavner study noted above showed that certain substitutions could be made in the thymopentin structure at the 2, 4 and 5 positions without adversely affecting activity. For example, the Lys at position 2 could successfully be replaced by a proline residue and less successfully with a D-Lys or α-aminoisobutyric acid (Aib). The Val at position 4 could be replaced by its D-isomer, Ala or sarcosine. The Tyr residue at position 5 could not be deleted, or replaced by Ala, but analogs with varying activities were obtained when it was replaced by Val, Phe, D-Tyr, 3-nitro-Tyr, 3-chloro-Tyr (but not 3-hydroxy-Tyr), His and Trp.

The relative simplicity of these results was superseded when the same group reported in 1986 on its attempts to synthesize biologically active analogs with enhanced stability to degradation. As the problem of short half-life was recognized, attempts were made to design analogs which would have a longer half-life in plasma. The report of this study, Heavner, G.A., et al., Peptides (1986) 7:1015-1019, reported the activity of a number of N-terminal acetylated analogs and C-terminal

amidated analogs on activity. Although the previous paper had shown that proline, Aib and D-Lys could be substituted in position 2, N-terminal acetylation of these peptides resulted in loss of activity for the Aib^2 analog and for the native thymopentin. On the other hand, the Pro^2 analog retained full activity. Analogously, perhaps, when the thymopentin peptide itself was amidated at the C-terminus activity was lost; however, activity was retained in the Pro^2 and Aib^2 analogs. For the thymopentin itself, however, a combination of N-terminal acetylation and C-terminal amidation resulted in an active molecule. This was not true for the Aib^2 analog.

The analogs reported in this paper, generally had enhanced half-life in serum as compared to thymopentin itself.

A comparison of the biological specificities of the splenin and thymopentin peptides at a molecular level was recently reported by Heavner, G.A., et al., Regulatory Peptides (1990) 27:257-262. The assay system used was the elevation of cGMP in two different human T-cell lines: CEM and MOLT-4. Thymopentin was capable of such stimulation for both types of T-cells whereas human thysplenin simulates cGMP only in MOLT-4. Various forms of the human analog of thysplenin (which substitutes Ala for the Glu of bovine thysplenin in the 34-position of the full-length peptide (3-position of the pentapeptide))

were used in the assay. Human "splenopentin" (splenin) having the sequence Ac-Arg-Lys-Ala-Val-Tyr-NH_2 was inactive with respect to the receptors on CEM cells but active with respect to MOLT-4 cells. This pattern was retained when the corresponding tetrapeptide missing the C-terminal Tyr or its Pro^2 analog was used. Substitution of phenylalanine for Tyr in the Pro^2 analog of thymopentin reversed the pattern activity.

The foregoing studies show that in general, minor modifications can be made to the pentapeptide sequence, and immunomodulatory activity in general can be retained, with the exception of the requirement for Asp, Glu or Ala at the 3-position and Arg at the 1-position, although shifts in specificity at the molecular level may occur.

Certain of the thymopentin analogs have also been made the subject of patent applications. Japanese application 80/46990, based on U.S. priorities of 12 April 1979 and 13 March 1980, discloses and claims a large genus of pentapeptides notably including Arg-D-Ala-Asp-Val-Tyr-NH_2. Japanese application J61/050998-A, laid open 7 August 1989, to Sloan-Kettering, discloses and claims derivatized forms of the pentapeptide wherein the side-chains of the various residues are modified. European application publication No. 282891, published 21 September 1988, discloses and claims "retroinverso" analogs of the pentapeptide and a tripeptide fragment. In these analogs, some of the peptide linkages are reversed.

There remains a need to design drugs having thymopentin activity which have acceptable half-lives in plasma and which retain the nontoxicity and effectiveness of the drug. The present invention is directed to such analogs wherein one or more of the peptide linkages of thymopentin or an active analog thereof is replaced by an isosteric nonpeptide linkage.

Disclosure of the Invention

The invention provides protease-resistant pseudopeptides which are effective in modulating the immune system and in treating immune diseases such as arthritis. They are useful in the treatment and control of diseases such as autoimmune and infectious diseases. These compounds retain the essential features of thymopentin, but contain at least one nonpeptide linkage between the amino acid residues which comprise the pentapeptide.

Accordingly, in one aspect, the invention is directed to pseudopeptides of the formula:

I II III IV

$$R\text{-}AA_1\text{--}AA_2\text{--}AA_3\text{--}AA_4\text{--}AA_5\text{-}R_1$$

wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a basic amino acid residue, a neutral/nonaromatic amino acid residue or proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid or glutamic acid residue, wherein the remaining carboxyl group may optionally be esterified with an alkyl group

(1-6C), or an alanine residue;

AA_4 is an L- or D-form of a neutral/nonaromatic amino acid residue;

AA_5 is an L- or D-form of a neutral/aromatic amino acid residue wherein one or more hydrogens of its aromatic portion can be substituted by NO_2 or halogen or is an L- or D-form of a neutral/nonpolar/large/ nonaromatic amino acid residue or is the N-alkylated (1-6C) form of the above;

R is acyl (1-6C), arylsulfonyl, alkylsulfonyl, arylalkylsulfonyl or alkoxycarbonyl group;

R_1 is -OH, $-NR_2R_3$ or $-OR_4$ wherein each of R_2 and R_3 is independently hydrogen or an alkyl group (1-6C) and R_4 is alkyl group (1-6C);

wherein at least one of the linkages numbered I-IV is a modified peptide linkage selected from the group consisting of $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2NH-$, and the remaining linkages are -CONH- or $-CON(CH_3)-$,

and the pharmaceutically acceptable salts thereof.

In a second aspect, the invention is directed to pseudopeptides of the formula:

I II III IV

$$R-AA_1--AA_2--AA_3--AA_4--AA_5-R_1$$

wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a basic amino acid residue, a neutral/nonaromatic amino acid residue or

proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid residue wherein the remaining carboxyl group may optionally be esterified with an alkyl group (1-6C);

AA_4 is an L- or D-form of a neutral/nonaromatic amino acid residue;

AA_5 is an L- or D-form of a neutral/aromatic amino acid residue wherein one or more hydrogens of its aromatic portion can be substituted by NO_2 or halogen or is an L- or D-form of a valine residue, or is an N-alkylated (1-6C) form of the above;

R is acyl (1-6C), arylsulfonyl, alkylsulfonyl, arylalkylsulfonyl or alkoxycarbonyl group;

R_1 is -OH, $-NR_2R_3$ or $-OR_4$ wherein each of R_2 and R_3 is independently hydrogen or an alkyl group (1-6C) and R_4 is alkyl group (1-6C);

wherein at least one of the linkages numbered I-IV is a modified peptide linkage selected from the group consisting of $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2NH-$, and the remaining linkages are -CONH- or $-CON(CH_3)-$,

and the pharmaceutically acceptable salts thereof.

In a third aspect, the invention is directed to pseudopeptides of the formula:

I II III IV

$$R\text{-}AA_1\text{--}AA_2\text{--}AA_3\text{--}AA_4\text{--}AA_5\text{-}R_1$$

wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a basic amino acid residue, a neutral/nonaromatic amino acid residue or proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid residue wherein the remaining carboxyl group may optionally be esterified with an alkyl group (1-6C);

AA_4 is an L- or D-form of an alanine or valine residue;

AA_5 is an L- or D-form of a neutral/aromatic amino acid residue wherein one or more hydrogens of its aromatic portion can be substituted by NO_2 or halogen or is an L- or D-form of a valine residue, or is an N-alkylated (1-6C) of the above;

R is acyl (1-6C), arylsulfonyl, alkylsulfonyl, arylalkylsulfonyl or alkoxycarbonyl group;

R_1 is -OH, $-NR_2R_3$ or $-OR_4$ wherein each of R_2 and R_3 is independently hydrogen or an alkyl group (1-6C) and R_4 is alkyl group (1-6C);

wherein at least one of the linkages numbered I-IV is a modified peptide linkage selected from the group consisting of $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2NH-$, and the remaining linkages are -CONH- or $-CON(CH_3)-$;

and the pharmaceutically acceptable salts thereof.

In a fourth aspect, the invention is directed to pseudopeptides of the formula:

I II III IV

$$R\text{-}AA_1\text{--}AA_2\text{--}AA_3\text{--}AA_4\text{--}AA_5\text{-}R_1$$

wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a basic amino acid residue, a neutral/nonaromatic amino acid residue or proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid residue wherein the remaining carboxyl group may optionally be esterified with an alkyl group (1-6C);

AA_4 is an L- or D-form of an alanine or valine residue;

AA_5 is an L- or D-form of a phenylalanine or tyrosine residue wherein one or more hydrogens of its aromatic portion can be substituted by NO_2 or halogen or is an L- or D-form of a valine residue, or is an N-alkylated (1-6C) of the above;

R is acyl (1-6C), arylsulfonyl, alkylsulfonyl, arylalkylsulfonyl or alkoxycarbonyl group;

R_1 is -OH, $-NR_2R_3$ or $-OR_4$ wherein each of R_2 and R_3 is independently hydrogen or an alkyl group (1-6C) and R_4 is alkyl group (1-6C);

wherein at least one of the linkages numbered I-IV is a modified peptide linkage selected from the group consisting of $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2NH-$, and the remaining linkages are -CONH- or $-CON(CH_3)-$,

and the pharmaceutically acceptable salts thereof.

In a fifth aspect, the invention is directed to

pseudopeptides of the formula:

I II III IV

$R\text{-}AA_1\text{--}AA_2\text{--}AA_3\text{--}AA_4\text{--}AA_5\text{-}R_1$

wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a lysine, alanine, α-aminoisobutyric acid, leucine, norleucine or proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid residue wherein the remaining carboxyl group may optionally be esterified with an alkyl group (1-6C);

AA_4 is an L- or D-form of an alanine or valine residue;

AA_5 is an L- or D-form of a phenylalanine or tyrosine residue wherein one or more hydrogens of its aromatic portion can be substituted by NO_2 or halogen or is an L- or D-form of a valine residue, or is an N-alkylated (1-6C) form of the above;

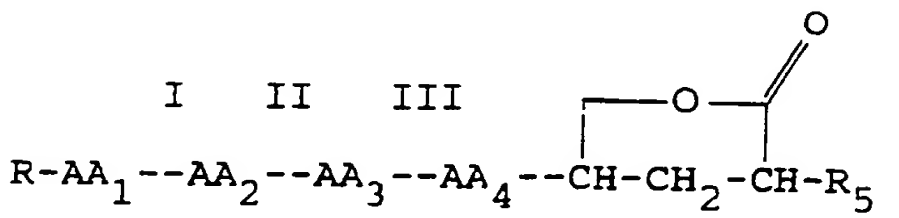
R is acyl (1-6C), arylsulfonyl, alkylsulfonyl, arylalkylsulfonyl or alkoxycarbonyl group;

R_1 is -OH, $-NR_2R_3$ or $-OR_4$ wherein each of R_2 and R_3 is independently hydrogen or an alkyl group (1-6C) and R_4 is alkyl group (1-6C);

wherein at least one of the linkages numbered I-IV is a modified peptide linkage selected from the group consisting of $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2NH-$, and the remaining linkages are -CONH- or $-CON(CH_3)-$,

and the pharmaceutically acceptable salts thereof.

In a sixth aspect, the invention is directed to pseudopeptides of the formula:



wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a lysine, alanine, α-aminoisobutyric acid, leucine, norleucine or proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid residue wherein the remaining carboxyl group may optionally be esterified with alkyl group (1-6C);

AA_4 is an L- or D-form of an alanine or valine residue;

R is an acyl group (1-6C) or an arylsulfonyl group, alkylsulfonyl group, arylalkylsulfonyl group or alkoxycarbonyl group;

R_5 is selected from the group consisting of a benzyl optionally substituted by NO_2 or halogen, 4-hydroxy-benzyl optionally substituted by NO_2 or halogen and isopropyl group, or is an N-alkylated (1-6C) form thereof;

wherein linkages numbered I-III are each independently -CONH- or $-CON(CH_3)-$;

and the pharmaceutically acceptable salts thereof.

The invention is also directed to pharmaceutical

compositions containing the compound of the present invention as active ingredient; these compositions are useful for effecting immunomodulation or for the treatment of arthritis. In another aspect, the invention is directed to methods to effect immunomodulation in a subject or to treat immune diseases such as arthritis using the compounds and compositions of the invention.

Brief Description of the Drawings

Figure 1 schematically outlines the classification of amino acids.

Modes for Carrying Out the Invention

The invention compounds are pseudopeptides containing five amino acid residues wherein at least one peptide bond linking these residues is substituted for by an isosteric linkage selected from the group set forth above. That is, in place of the CONH linkage ordinarily coupling the amino acid residues of the compound of the present invention, these functionalities (derived from the amino acid residues) are modified so as to obtain the isosteres. The resulting compounds have enhanced stability to protease degradation, and are therefore more effective therapeutic and prophylactic agents *in vivo*.

Compounds of the Invention

The compounds of the formula

$$\overset{I}{R\text{-}AA_1} - \overset{II}{AA_2} - \overset{III}{AA_3} - \overset{IV}{AA_4} - AA_5\text{-}R_1 \qquad (1)$$

are defined in terms of specifying amino acid residues

AA_1-AA_5. In these definitions, AA_2 is defined as a basic amino acid, a neutral/nonaromatic amino acid, or proline residue; AA_4 is a neutral/nonaromatic amino acid residue, and AA_5 is a neutral/aromatic amino acid or a neutral/nonpolar/large/nonaromatic amino acid or derivatives or substituted forms thereof. These classifications are explained as follows:

Amino acid residues can be generally subclassified into four major subclasses as follows:

Acidic: The residue has a negative charge due to loss of H ion at physiological pH and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium at physiological pH.

Basic: The residue has a positive charge due to association with H ion at physiological pH and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium at physiological pH.

Neutral/nonpolar: The residues are not charged at physiological pH and the residue is repelled by aqueous solution so as to seek the inner positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium. These residues are also designated "hydrophobic" herein.

Neutral/polar: The residues are not charged at physiological pH, but the residue is attracted by aqueous solution so as to seek the outer positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium.

It is understood, of course, that in a statistical collection of individual residue molecules some molecules will be charged, and some not, and there will be an attraction for or repulsion from an aqueous medium to a greater or lesser extent. To fit the definition of "charged", a significant percentage (at least approximately 25%) of the individual molecules are charged at physiological pH. The degree of attraction or repulsion required for classification as polar or nonpolar is arbitrary and, therefore, amino acids specifically contemplated by the invention have been specifically classified as one or the other. Most amino acids not specifically named can be classified on the basis of known behavior.

Amino acid residues can be further subclassified as cyclic or noncyclic, and aromatic or nonaromatic, self-explanatory classifications with respect to the side chain substituent groups of the residues, and as small or large. The residue is considered small if it contains a total of 4 carbon atoms or less, inclusive of the carboxyl carbon. Small residues are, of course, always nonaromatic.

For the naturally occurring protein amino acids, subclassification according to the foregoing scheme is as follows.

Acidic: Aspartic acid and Glutamic acid;

Basic/noncyclic: Arginine, Lysine;

Basic/cyclic: Histidine;

Neutral/polar/small: Glycine, Serine, Cysteine;

Neutral/polar/large/nonaromatic: Threonine, Asparagine, Glutamine;

Neutral/polar/large/aromatic: Tyrosine;

Neutral/nonpolar/small: Alanine;

Neutral/nonpolar/large/nonaromatic: Valine, Isoleucine, Leucine, Methionine;

Neutral/nonpolar/large/aromatic: Phenylalanine, and Tryptophan.

The gene-encoded amino acid proline, although technically within the group neutral/nonpolar/large/cyclic and nonaromatic, is a special case due to its known effects on the secondary conformation of peptide chains, and is not, therefore, included in this defined group.

Certain commonly encountered amino acids, which are not encoded by the genetic code, include, for example, beta-alanine (beta-Ala), or other omega-amino acids, such as 3-aminopropionic, 4-aminobutyric and so forth, alpha-aminoisobutyric acid (Aib), sarcosine (Sar), ornithine (Orn), citrulline (Cit), t-butylalanine

(t-BuA), t-butylglycine (t-BuG), N-methylisoleucine (N-MeIle), phenylglycine (Phg), and cyclohexylalanine (Cha), norleucine (Nle), cysteic acid (Cya) and methionine sulfoxide (MSO). These also fall conveniently into particular categories.

Based on the above definition,

Sar, beta-Ala and Aib are neutral/nonpolar/small;

t-BuA, t-BuG, N-MeIle, Nle and Cha are neutral/nonpolar/large/nonaromatic;

Orn is basic/noncyclic;

Cya is acidic;

Cit, Acetyl Lys, and MSO are neutral/polar/large/nonaromatic; and

Phg is neutral/nonpolar/large/aromatic.

In addition to the amino acids classified above, the derivatives of them can be included, for example, 2-amino-3-(3-nitro-4-hydroxyphenyl)propionic acid, 2-amino-3-(3-fluoro-4-hydroxyphenyl)propionic acid 2-amino-3-(3-chloro-4-hydroxyphenyl)propionic acid, 2-amino-3-(3-bromo-4-hydroxyphenyl)propionic acid, 2-amino-3-(3-iodo-4-hydroxyphenyl)propionic acid and the like.

The various omega-amino acids are classified according to size as neutral/nonpolar/small (beta-ala, i.e., 3-aminopropionic, 4-aminobutyric) or large (all others).

Other amino acid substitutions of those encoded in the gene can also be included in peptide compounds within

the scope of the invention and can be classified within this general scheme.

Preferred embodiments for AA_2 include the lysyl residue of the known thymopentin, and also residues of proline, norleucine, leucine, alanine, α-aminoisobutyric acid and N-alkylated form thereof.

A preferred embodiment of AA_3 is an aspartyl residue wherein the remaining carboxyl group may optionally be esterified with an alkyl group (1-6C).

Preferred embodiments of AA_4 include the valyl residue of thymopentin, as well as the residues of alanine, isoleucine and leucine.

Preferred embodiments of AA_5 include the residue of phenylalanine and tyrosine (which occurs in thymopentin) wherein hydrogen atoms of their aromatic part can be substituted by NO_2 group or halogen, and valine and N-alkylated forms thereof.

The α-amino group at the N-terminus of the compounds of formula (1) can also be acylated, arylsulfonylated, alkylsulfonylated, arylalkylsulfonylated or alkoxy carbonylated. The acylating groups are corresponding 1-6C acyl moieties including formyl, acetyl, pentanyl, isobutyryl, and the like. The arylsulfonylating groups are benzensulfonyl, o-toluene-sulfonyl, m-toluene-sulfonyl, p-toluene-sulfonyl, xylenesulfonyl biphenylsulfonyl, naphthalenesulfonyl, and the like. The alkylsulfonylating groups are methylsulfonyl,

ethylsulfonyl, propylsulfonyl, butylsulfonyl, pentylsulfonyl, hexylsulfonyl and the like. The arylalkylsulfonylating groups are benzylsulfonyl, phenylethylsulfonyl, phenylpropylsulfonyl, phenylbutylsulfonyl, phenylpentylsulfonyl, phenylhexylsulfonyl, and the like. The alkoxy carbonylating groups are ethoxy carbonyl, butoxy carbonyl, i-pentoxy carbonyl, benzyloxy carbonyl, and the like.

At the C-terminus of the compounds of formula (1), the carboxyl group may be in the underviatized form or may be amidated or esterified; in the underivatized form the carboxyl may be as a free acid or a salt, preferably a pharmaceutically acceptable salt.

The nitrogen atom of the amido group, covalently bound to the carbonyl carbon at the C-terminus, will be NH_2, -NHR, or NRR', wherein R and R' are straight or branched chain alkyl of 1-6C, such alkyls are 1-6C straight- or branched-chain saturated hydrocarbon residues, such as methyl, ethyl, propyl, isopropyl, butyl, pentyl, hexyl, isopentyl, and the like. Representatives of such amido groups are: $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, $-NHCH_2CH_3$, $-NHCH_2CH(CH_3)_2$, and $-NHCH_2CH(CH_3)CH_2CH_3$, among others. The esterified forms are methyl ester, ethyl ester, propyl ester, butyl ester, pentyl ester, hexyl ester, and the like.

The substituted-for peptide linkage of the invention

is selected from the group consisting of $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2NH-$. Preferred embodiments of this modified linkage include $-COCH_2-$ and $-CH(OH)CH_2-$. Especially preferred is $-COCH_2-$.

Although more than one substitution in linkages can be made, preferred embodiments of the compounds of the invention are those wherein only two such linkages are substituted, and most preferably one peptide linkage is substituted, and in all of the cases the remaining linkages except for the modified linkages are -CONH- or $-CON(CH_3)-$.

As indicated in the Heavner group papers, various combinations of substitutions lead to relatively unpredictable results in activity; hard and fast guidelines, other than those already established, are difficult to lay down. However, the efficacy of the resulting pseudopeptide can be readily ascertained, and only those peptides exhibiting activity to modulate the immune system are part of the invention.

Assay Systems

The efficacy of the various pseudopeptides of the invention can be determined experimentally using a number of in vitro model systems. Competition for T-cell receptors with labeled thymopentin or thymopoietin is one convenient way to ascertain the ability of the pseudopeptide to bind to target cells. Alternatively or in addition, the ability of the pseudopeptides to

stimulate cyclic GMP production in targeted T-cells can also be determined. Both of these determinations can be made according to the methods of Heavner, G.A., et al., Arch Biochem Biophys (1985) 242:248-255, cited above.

For these assays, CEM cells are obtained from the American Type Culture Collection (Rockville, MD) and subcultured in flasks at 2×10^5 cells per flask in PRMI-1640 in 20% serum, and stored frozen at 3×10^6 cells/mL. For the cGMP assay, final concentration of CEM cells was adjusted to 1×10^7/mL in RPMI medium. After equilibration the compound to be tested is added for a two minute incubation. The reaction is terminated by addition of ice cold TCA, the samples are frozen-thawed three times, and the TCA is removed by ether extraction. After lyophilization, the samples are resuspended in acetate buffer and the cGMP levels are determined by RIA.

Typical competitive thymopentin binding assays employ tritiated thymopentin with a specific activity of 37 Ci/mmole. CEM cells are adjusted to a final concentration of 5×10^6 cells/0.1 mL of binding buffer. Dilutions of thymopentin or thymopentin analogs are added to the cells over a concentration range of 10^{-6} to 10^{-3} M. The radiolabeled ^{3}H-thymopentin is then added (548,000 cpm), and the tubes are incubated at 25°C for 30 minutes. The cells are then diluted with 1 mL of ice cold binding buffer, washed two times by centrifugation, and resuspended. Radioactivity is determined by

scintillation counting.

Corroborative results are obtainable in alternate assays testing the ability of a sample compound to enrich a population of prethymocytes for the presence of the Thy^+-1 thymocyte marker. To conduct this assay, prethymocytes are prepared from isolated spleens of nude mice.

For spleen cell preparation, whole spleens are collected from Balb/C, nu/nu mice, minced and pooled in RPMI-1640. The thymocytes are collected by centrifugation in Ficoll-Hypaque and then resuspended at 1×10^6 cells/mL in RPMI-1640.

The spleen cells are incubated with test compound at various concentrations for 4.5 hours at 37°C, washed in PBS containing 0.1% azide, and then treated with FITC-labelled antibody at 4°C for 30 minutes. The cells are then washed by centrifugation through heat-inactivated fetal bovine serum and then fixed for analysis with a fluorescence-activated cell-sorter to determine the number of cells containing Thy^+-1.

The humoral response is measured by ELISA. The ELISA is conducted by assay coating Immulon II microtiter wells at 4°C overnight using 100 µl of a 10 µg/mL solution of bovine type II collagen. The plates are then washed with buffered saline containing the detergent Tween 20 at a concentration of 0.05%. Nonspecific binding sites are blocked by addition of a 2% solution of

bovine serum albumin in buffered saline. The plates are then washed, and the mouse serum dilutions added to the wells. Recognition of antibody binding is carried out using peroxidase conjugated goat anti-mouse IgG antibody. Enzyme-dependent color development is quantified by reading the optical density at 490 nm using an automated ELISA reader.

Preferred Embodiments

The nomenclature used to describe the pseudopeptides of the present invention follows the conventional practice where the N-terminal amino group is assumed to be to the left and the carboxy group to the right of each amino acid in the peptide. In the formulas representing selected specific embodiments of the present invention, the amino- and carboxy-terminal groups, although often not specifically shown, will be understood to be in the form they would assume at physiological pH values, unless otherwise specified. Thus, the N-terminal H^+_2 and C-terminal -O- at physiological pH are understood to be present though not necessarily specified and shown, either in specific examples or in generic formulas. In the peptides shown, each encoded residue, where appropriate, is represented by a single- or triple-letter designation, corresponding to the trivial name of the amino acid, in accordance with the following conventional list:

Amino Acid	One-Letter Symbol	Three-Letter Symbol
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
Glutamic acid	E	Glu
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val

The amino acids not encoded genetically are abbreviated as indicated in the discussion above.

In the specific peptides shown in the present application, the L-form of any amino acid residue having an optical isomer is intended unless otherwise expressly

indicated by a D or a dagger superscript (†). While the residues of the invention peptides are normally in the natural L optical isomer form, one or two, preferably one, amino acid may be replaced with the optical isomer D-form.

Free functional groups on the side chains of the amino acid residues can also be modified by amidation, acylation or other substitution, which can, for example, change the solubility of the compounds without affecting their activity.

In formulas directly indicating amino acid sequence, the inclusion of (k) indicates the linkage $-COCH_2-$ as a replacement for -CONH-; (CHOH) indicates $-CH(OH)CH_2-$ as a replacement for -CONH-; "R" indicates $-CH_2NH-$ as a replacement for -CONH-. Acetylation at the N-terminus is indicated by N-Ac-; amidation at the C-terminus by $-CONH_2$. Some preferred embodiments of the invention compounds, using this notation, are shown in Table 1.

Table 1

1. Arg-Lys-Asp-Val(k)Phe
2. Arg-Lys-Asp-Val(k)Tyr
3. Arg-Pro-Asp-Val(k)Phe
4. Arg-Nle-Asp-Val(k)Phe
5. Arg-Lys-Asp-Ala(k)Phe
6. Arg-Lys-Asp-Val(k)Val
7. Arg-Aib-Asp-Val(k)Phe

8. Arg-Lys-Asp-Val(k)Phe-$CONH_2$
9. N-Ac-Arg-Lys-Asp-Val(k)Phe
10. N-Ac-Arg-Lys-Asp-Val(k)Phe-$CONH_2$
11. Arg-Lys-Asp-Val(CHOH)Phe
12. Arg-N-MeLys-Asp-Val(k)Phe
13. Arg-Nle-Asp-Ala(k)Phe
14. Arg-Nle-Asp-Val(k)Tyr
15. Arg-Nle-Asp-Val(k)Phe-$CONH_2$
16. Arg-Nle-Asp-Val(CHOH)Phe
17. Arg-N-MeNle-Asp-Val(k)Phe
18. Arg-Leu-Asp-Val(k)Phe
19. N-Ac-Arg-Pro-Asp-Val(k)Phe
20. N-Ac-Arg-Nle-Asp-Val(k)Phe
21. Arg-Lys†-Asp-Val(k)Phe
22. N-Benzoyl-Arg-Nle-Asp-Val(k)Phe
23. N-Tosyl-Arg-Nle-Asp-Val(k)Phe
24. N-MeOCO-Arg-Nle-Asp-Val(k)Phe
25. Arg-Nle-Asp-Val(k)Val
26. Arg-N-MeLeu-Asp-Val(k)Phe
27. Arg(k)Lys-Asp-Val-Phe
28. Arg(k)Nle-Asp-Val-Phe
29. Arg(k)Nle-Asp-Val(k)Phe
30. N-Ac-Arg(k)Nle-Asp-Val-Phe
31. N-Ac-Arg(k)Nle-Asp-Val-Phe-$CONH_2$
32. Arg(k)Nle-Asp-Val-Phe-$CONH_2$
33. Arg(k)Nle-Asp-Ala-Phe
34. Arg(k)Nle-Asp-Val-Val

35. Arg(k)Nle-Asp-Val(k)Val

36. Arg(CHOH)Nle-Asp-Val-Phe

37. Arg(CHOH)Lys-Asp-Val-Phe

38. Arg(k)Lys-Asp-Val-N-MePhe

39. Arg(k)Nle-Asp-Val-N-MePhe

40. Arg(k)Nle-Asp-Val†-Phe

41. Arg-Lys-Asp(k)Val-Phe

42. Arg-Nle-Asp(k)Val-Phe

43. Arg-Pro-Asp(k)Val-Phe

44. Arg-Aib-Asp(k)Val-Phe

45. Arg(k)Nle-Asp(k)Val-Phe

46. N-Ac-Arg-Lys-Asp(k)Val-Phe

47. N-Ac-Arg-Pro-Asp(k)Val-Phe

48. N-Ac-Arg-Pro-Asp(k)Val-Phe-$CONH_2$

49. Arg-Pro-Asp(k)Val-Phe-$CONH_2$

50. Arg-Lys-Asp(k)Ala-Phe

51. Arg-Lys-Asp(k)Val-Tyr

52. Arg-Lys-Asp(k)Val-Phe-$CONH_2$

53. Arg-Lys-Asp(k)Val-D-Tyr

54. Arg-NMeLys-Asp(k)Val-Phe

55. Arg-Nle-Asp(k)Val-Tyr

56. Arg(R)Lys-Asp-Val-Phe

57. Arg(R)Lys-Asp-Val(k)Phe

58. N-Ac-Arg(R)Lys-Asp-Val(k)Phe

59. N-Ac-Arg(R)Lys-Asp(k)Val-Phe

60. Arg-Ala-Asp-Val(k)Phe

61. Arg-Nle-Asp(OMe)-Val(k)Phe(OMe)

62. Arg-NMeNle-Asp-Val(k)Val

63. Arg-Lys-Asp(OMe)(k)Val-Phe(OMe)

A notation specifically reflecting features of the various pseudopeptides of the invention can be, if desired, adapted to show their modification from thymopentin, which is abbreviated THP. Thymopentin in humans is a pentapeptide of the formula Arg-Lys-Asp-Val-Tyr, in positions 1-5. The pseudopentapeptides are designated by modifications of this sequence. Thus, for example, P^2-THP denotes the pseudopentapeptide wherein the Lys at position 2 is replaced by a proline. As a single letter amino acid code is used, the D-enantiomer will be denoted using a superscript cross. Thus, $D^{\dagger 3}$-THP represents a form in which the Asp at position 3 is replaced by its D-enantiomer.

Acylation at the N-terminus is noted as a prefix to the formula. Thus, for example, Ac-P^2-THP represents acetylated thymopentin where the amino acid at position 2 is proline; CH_3CH_2CO-F^5-THP refers to propionyl thymopentin where an amino acid at position 5 is phenylalanine. C-terminal amidation is noted as a suffix. Thus, A^4-THP-$NHCH_3$ refers to an analog wherein the amino acid at position 4 is alanine and the tyrosine at position 5 is amidated with methylamine. N-MeNle^{2}F^5-THP refers to an analog wherein the amino acid at position 2 is norleucine which is alkylated with a

methyl group and the amino acid at position 5 is phenylalanine. $D^3(OMe)F^5(OMe)$-THP refers to an analog wherein the aspartic acid at position 3 is esterified with a methyl group and the amino acid at position 5 is phenylalanine which is also esterified with a methyl group.

As all of the pseudopeptides of the invention contain at least one bond which is a substitute for the ordinary peptide linkage; this is indicated by a Roman numeral before the name. Thus, a Roman numeral in parentheses indicates the position of the substituted bond. As $-COCH_2-$ is a preferred embodiment of the substitution, if nothing else is indicated, this is the substituted bond at the indicated location. However, if a different embodiment of the substitution is intended, this is placed in parentheses after the designation, as indicated in compound 6 in Table 2 below. This terminology is used to indicate the location of the substituted bond and the alterations in the basic structure:

I II III IV

R - K - D - V - Y

Representative invention compounds using this notation are shown in Table 2.

Table 2

1.	(IV)	F^5-THP
2.	(IV)	P^2F^5-THP

3.	(IV)	$Nle^{2}F^{5}$-THP
4.	(IV)	$A^{4}F^{5}$-THP
5.	(IV)	Ac-F^{5}-THP
6.	(IV)	F^{5}-THP ($CHOHCH_2$)
7.	(IV)	$Nle^{2}A^{4}F^{5}$-THP
8.	(IV)	$Nle^{2}F^{5}$-THP ($CHOHCH_2$)
9.	(IV)	N-Me$Nle^{2}F^{5}$-THP
10.	(IV)	$L^{2}F^{5}$-THP
11.	(IV)	Ac-$P^{2}F^{5}$-THP
12.	(IV)	Ac-$Nle^{2}F^{5}$-THP
13.	(IV)	$K^{\dagger 2}F^{5}$-THP
14.	(IV)	$Nle^{2}V^{5}$-THP
15.	(IV)	N-Me$L^{2}F^{5}$-THP
16.	(IV)	$A^{2}F^{5}$-THP
17.	(I)	F^{5}-THP
18.	(I)	$Nle^{2}F^{5}$-THP
19.	(I)	$Nle^{2}F^{5}$-THP-NH_2
20.	(I)	$Nle^{2}V^{\dagger 4}F^{5}$-THP
21.	(III)	F^{5}-THP
22.	(III)	$Nle^{2}F^{5}$-THP
23.	(III)	$P^{2}F^{5}$-THP
24.	(III)	$A^{4}F^{5}$-THP
25.	(III)	Ac-F^{5}-THP
26.	(III)	$P^{2}F^{5}$-THP-NH_2
27.	(III)	THP
28.	(III)	N-Me$K^{2}F^{5}$-THP
29.	(III)	Nle^{2}-THP

30.	(I)	F^5-THP (CH_2NH)
31.	(IV)	N-MeNle^2V^5-THP
32.	(IV)	Nle^2D^3(OMe)F^5(OMe)-THP
33.	(III)	D^3(OMe)F^5(OMe)-THP

Synthesis

The peptide-linked portions of the pseudopepetides of the present invention can be synthesized chemically by means well known in the art such as, e.g., solid-phase peptide synthesis. The synthesis is commenced from the carboxy-terminal end of the peptide using an α-amino protected amino acid. t-Butyloxycarbonyl (Boc) protective groups can be used for all amino groups even though other protective groups are suitable. For example, Boc-Asp-OH, Boc-Val-OH, Boc-Phe-OH, Boc-Arg-OH, Boc-Nle-OH or Boc-Tyr-OH (i.e., selected carboxy-terminal amino acids) can be esterified to chloro-methylated polystyrene resin supports. The polystyrene resin support is preferably a copolymer of styrene with about 0.5 to 2% divinyl benzene as a cross-linking agent which causes the polystyrene polymer to be completely insoluble in certain organic solvents. See Stewart, et al., Solid-Phase Peptide Synthesis (1969), W.H. Freeman Co., San Francisco, and Merrifield, J Am Chem Soc (1963) 85:2149-2154. these and other methods of peptide synthesis are also exemplified by U.S. Patents No. 3,862,925; 3,842,067; 3,972,859; and 4,105,602.

The synthesis may use manual techniques or automatic, employing, for example, an Applied BioSystems 430A Peptide Synthesizer (Foster City, California) or a Biosearch SAM II automatic peptide synthesizer (Biosearch, Inc., San Rafael, California), following the instructions provided in the instruction manual supplied by the manufacturer.

In forming the amidated forms of the invention compounds, the analog compounds can be synthesized directly, for example, by using Boc-AA_x-pMBHA-Resin or Doc-AA_x-BHA-Resin, wherein AA_x is the selected carboxy-terminal amino acid of the N-terminal compound as described in further detail below. Alternatively, these forms can be chemically or enzymatically amidated subsequent to peptide synthesis using means well known to the art, or prepared by standard solution-phase peptide synthesis protocols.

In the pseudopeptides of the invention, at least one amide linkage (-CONH-) within the pentapeptide must be replaced with another linkage which is an isostere, such as $-CH_2NH-$, $-COCH_2-$ and $-CH(OH)CH_2-$, by methods known in the art. The following references describe preparation of peptide analogs which include these alternative-linking moieties: Spatola, A.F., Vega Data (March 1983), Vol. 1, Issue 3, "Peptide Backbone Modifications" (general review); Spatola, A.F., in "Chemistry and Biochemistry of Amino Acids Peptides and

Proteins," B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983) (general review); Morley, J.S., Trends Pharm Sci (1980) pp. 463-468 (general review); Hudson, D., et al., Int J Pept Prot Res (1979) 14:177-185 ($-CH_2NH-$, $-CH_2CH_2-$); Almquist, R.G., et al., J Med Chem (1980) 23:1392-1398 ($-COCH_2-$); Jennings-White, C., et al., Tetrahedron Lett (1982) 23:2533 ($-COCH_2-$); Szelke, M., et al., European Patent Application EP 45665 (1982) CA:97:39405 (1982) ($-CH(OH)CH_2-$); and Holladay, M.W., et al., Tetrahedron Lett (1983) 24:4401-4404 ($-CH(OH)CH_2-$). Particularly preferred are $-COCH_2-$ and $-CH(OH)CH_2-$.

The examples hereinbelow further illustrate methods to synthesize the various pseudopeptides of the invention. The intermediates which are constructed in accordance with the present disclosure during the course of synthesizing the present analog compounds are themselves novel and useful compounds and are thus within the scope of the invention.

Administration and Use

Compounds of the present invention are immunomodulators and are helpful in treating immune diseases such as arthritis in the intact mammal.

Thus these compounds, and compositions containing them, can find use as therapeutic agents in the treatment of various immune-system conditions such as congenital immunodeficiency (e.g., Digeorge Syndrome); acquired

immunodeficiency (e.g., postburn, postsurgery or postradiation damage and AIDS); autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis); allergic diseases (e.g., atopic dermatitis); inflammation; acute, chronic or recurrent infections by fungal, mycoplasma or virus (e.g., herpes, leprosy); sarcoidosis and psoriasis.

The present invention also provides compositions containing an effective amount of compounds of the present invention, including the nontoxic addition salts, amides and esters thereof, which may, alone, serve to provide the above-recited therapeutic benefits. Such compositions can also be provided together with physiologically tolerable liquid, gel or solid diluents, adjuvants and excipients.

These compounds and compositions can be administered to mammals for veterinary use, such as with domestic animals, and clinical use in humans in a manner similar to other therapeutic agents. In general, the dosage required for therapeutic efficacy will range from about 0.01 to 50,000 µg/kg, more usually 0.1 to 10,000 µg/kg of the host body weight. Alternatively, dosages within these ranges can be administered by constant infusion over an extended period of time until the desired therapeutic benefits have been obtained.

Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions;

solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active ingredient is often mixed with diluents or excipients which are physiologically tolerable and compatible with the active ingredient. Suitable diluents and excipients are, for example, water, saline, dextrose, glycerol, or the like, and combinations thereof. In addition, if desired, the compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, stabilizing or pH-buffering agents, and the like.

The compositions are conventionally administered parenterally, by injection, for example, either subcutaneously or intravenously. Additional formulations which are suitable for other modes of administration include suppositories, intranasal aerosols, and, in some cases, oral formulations. For suppositories, traditional binders and excipients may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1% to 2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions,

suspensions, tablets, pills, capsules, sustained-release formulations, or powders, and contain 1% to 95% of active ingredient, preferably 1% to 10%.

The peptide compounds may be formulated into the compositions as neutral or salt forms. Pharmaceutically acceptable nontoxic salts include the acid addition salts (formed with the free amino groups) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or organic acids such as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The compounds of the invention also can be administered alone or as mixtures and/or in conjunction with additional pharmaceuticals which modify their effects, such as modulators of the splenin class.

Compounds of the present invention can also be used for preparing antisera for use in immunoassays employing labeled reagents, usually antibodies. Conveniently, the polypeptides can be conjugated to an antigenicity-conferring carrier, if necessary, by means of dialdehydes, carbodiimide or using commercially available linkers. These compounds and immunologic reagents may be labeled with a variety of labels such as chromophores,

fluorophores such as, e.g., fluorescein or rhodamine, radioisotopes such as ^{125}I, ^{35}S, ^{14}C, or ^{3}H, or magnetized particles, by means well known in the art.

These labeled compounds and reagents, or labeled reagents capable of recognizing and specifically binding to them, can find use as, e.g., diagnostic reagents. Samples derived from biological specimens can be assayed for the presence or amount of substances having a common antigenic determinant with compounds of the present invention. In addition, monoclonal antibodies can be prepared by methods known in the art, which antibodies can find therapeutic use, e.g., to neutralize over-production of immunologically related compounds in vivo.

The following examples are intended to illustrate but not limit the invention. In the notation used, when the sequence of the compound of Formula (1) is spelled out, "(k)" denotes the $COCH_2$ linkage.

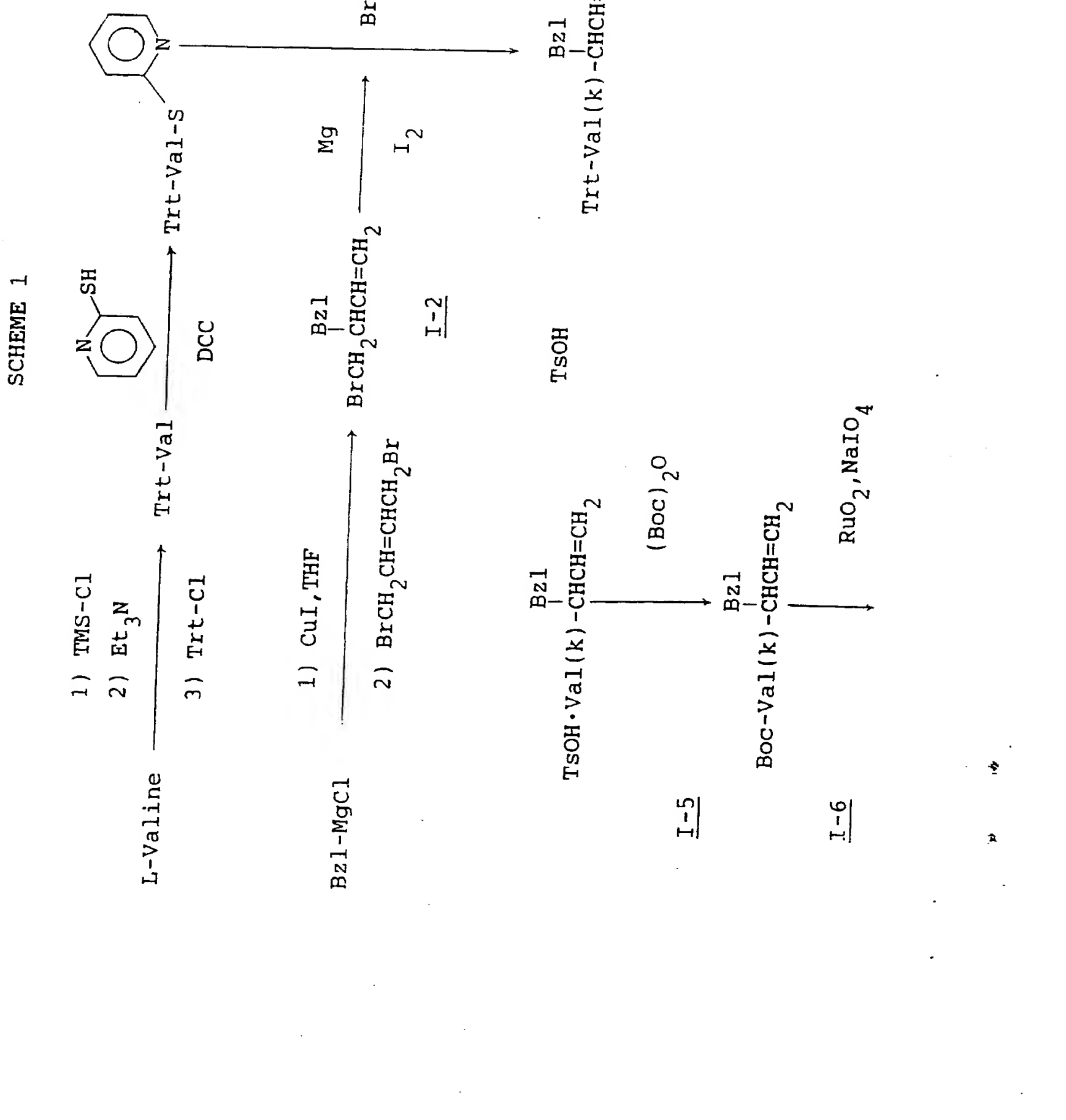
Each of Examples 1 to 3, 5 to 13, and 15 to 21 represents the synthesis of a compound of Formula (1) which has a $-COCH_2-$ linkage at peptide bond IV, I or III. Example 4 represents the synthesis of a compound of formula (1) which has a $-CHOHCH_2-$linkage at peptide bond IV. Example 22 represents the synthesis of a compound of formula (1) which has a $-CH_2NH-$ linkage at peptide bond I. In general, these syntheses can be seen in Reaction Schemes 1 to 13 and 15 to 22.

The compounds of this invention can also be prepared

by standard solution-phase peptide synthesis protocols. Example 14 represents the synthesis of a compound of Formula (1) which has a $-COCH_2-$ linkage at peptide bond IV by solution-phase peptide synthesis protocols. Example 23 and 24 represent the synthesis of a compound of Formula (1) which has a $-COCH_2-$ linkage at peptide bond III by solution-phase peptide synthesis protocols. These approaches are seen in Reaction Schemes 14, 23 and 24, respectively.

The procedures set forth in the examples more specifically delineate the conditions for reaction and so forth. The general methods for carrying out the indicated steps in Reaction Schemes 1-24 are well known to those of ordinary skill in the art.

SCHEME 1



SCHEME 1 (Continued)

Boc-Val(k)Phe-OH

1-7

$CsHCO_3$

$MeOH, H_2O$

Boc-Val(k)Phe-OCs

$ClCH_2$-Resin

Boc-Val(k)Phe-O-CH_2-Resin

I-8

1) TFA
2) iPrOH(Wash)
3) Boc-Lys(ClZ)-Asp(cHex)-OBt ← DCC / HOBt — Boc-Lys(ClZ)-Asp(cHex)-OH

I-9

Boc-Lys(ClZ)-Asp(cHex)-Val(k)Phe-O-CH_2-Resin

1) TFA
2) Wash
3) Boc-Arg(Ts)-OBt

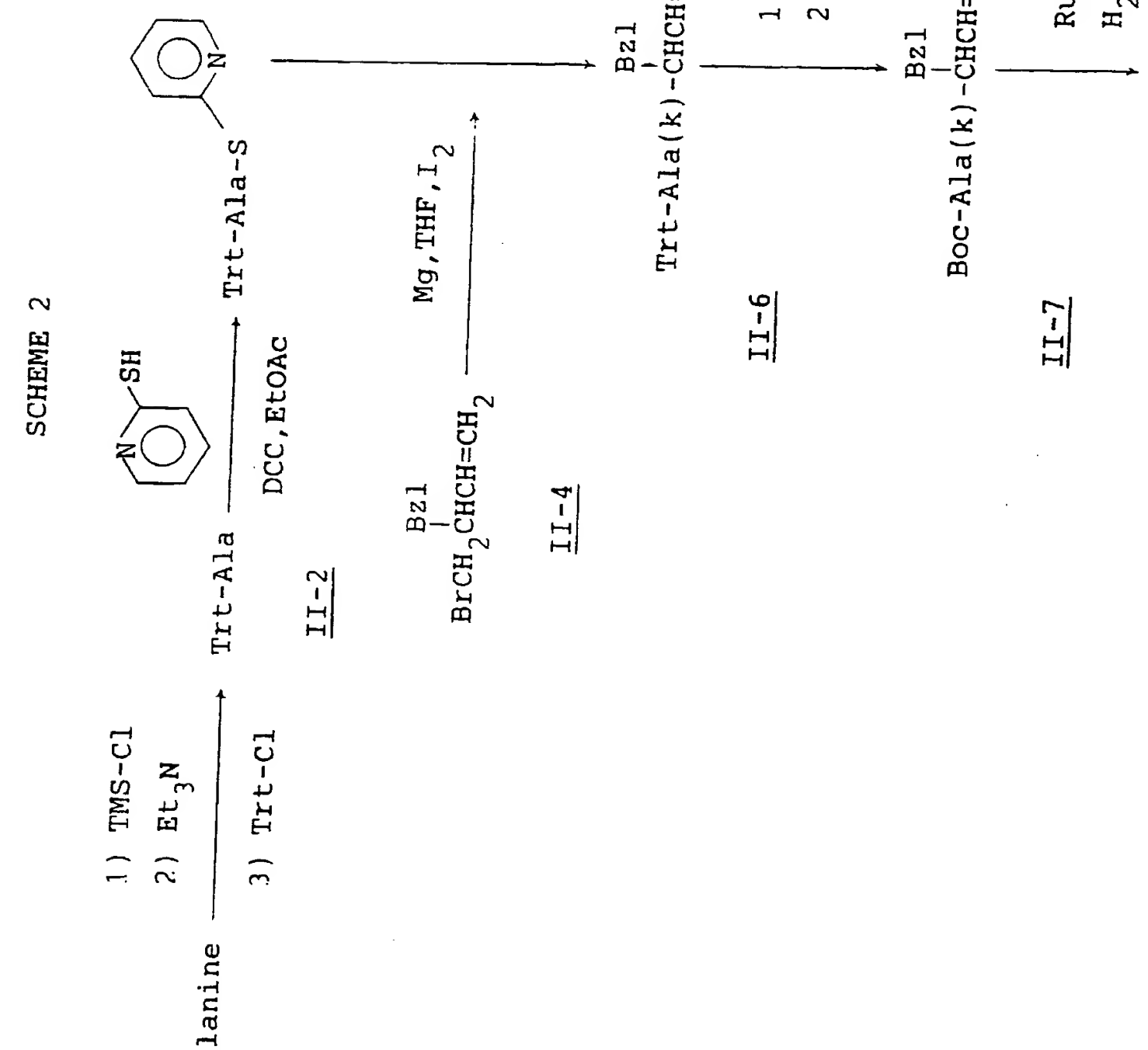
Boc-Arg(Ts)-Lys(ClZ)-Asp(cHex)-Val(k)Phe-O-CH_2-Resin

1) TFA
2) Wash
3) HF
4) Purification

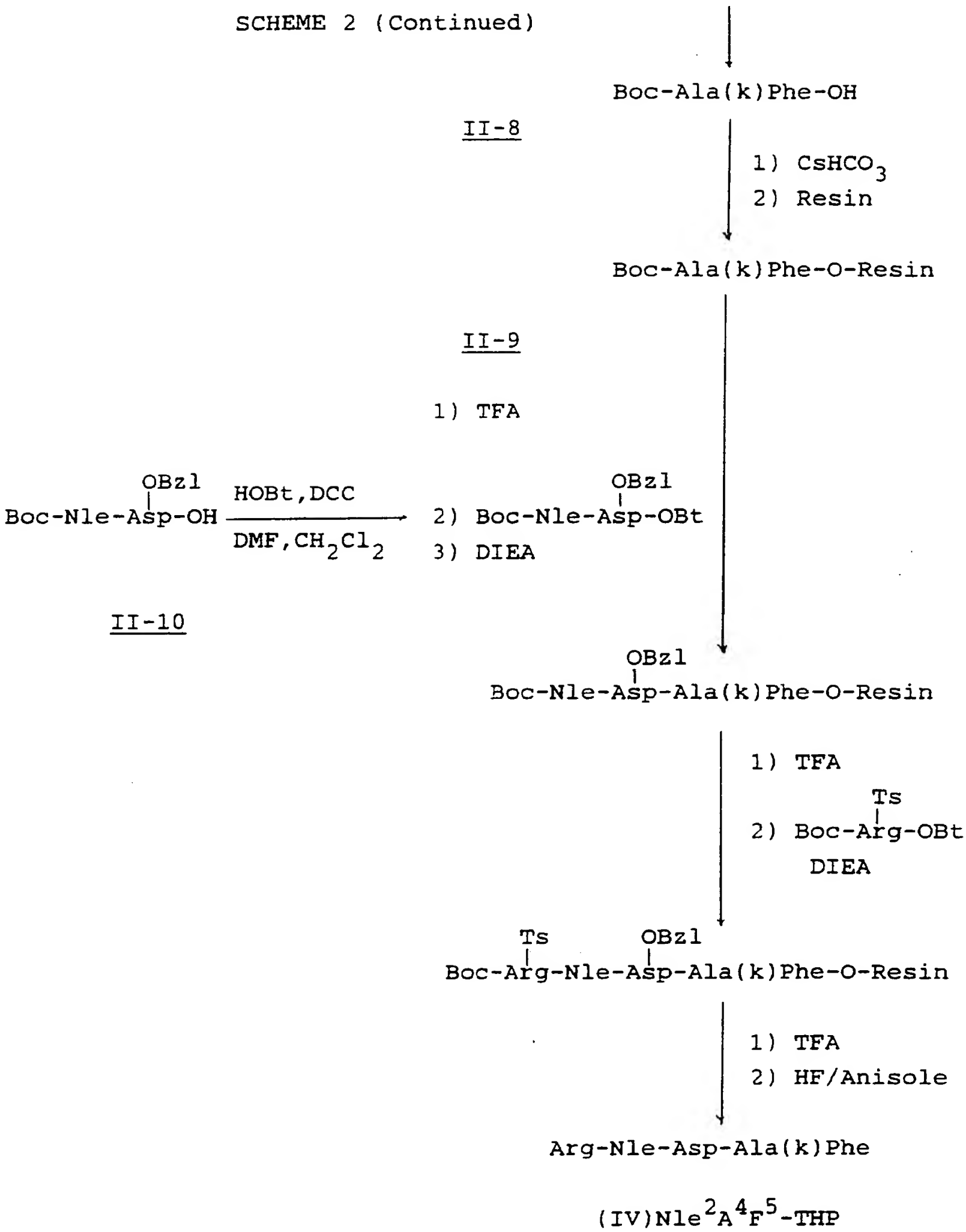
Arg-Lys-Asp-Val(k)Phe

(IV)F^5-THP

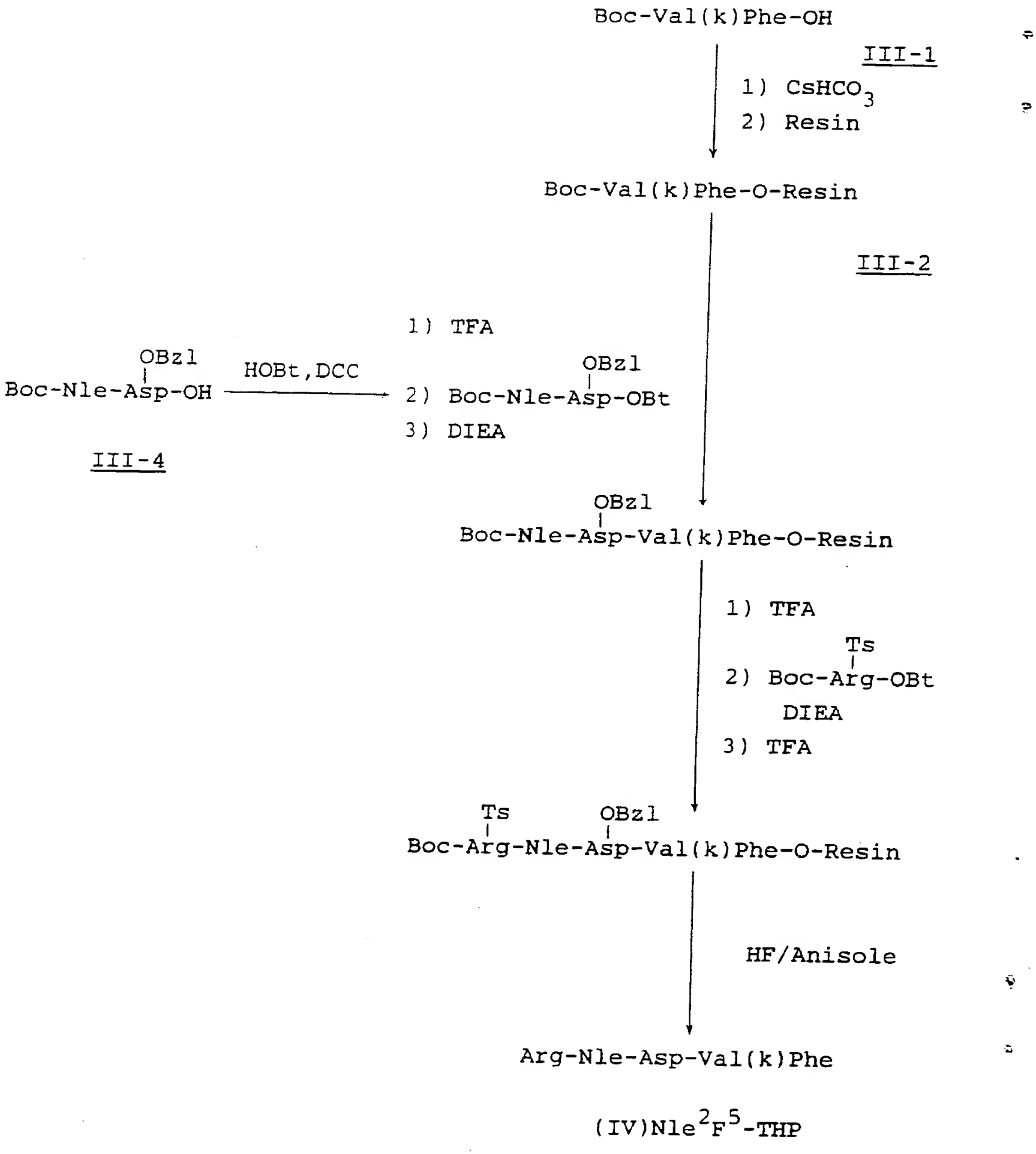
SCHEME 2



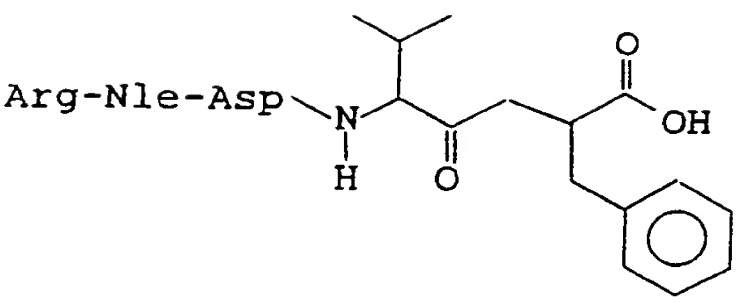
SCHEME 2 (Continued)



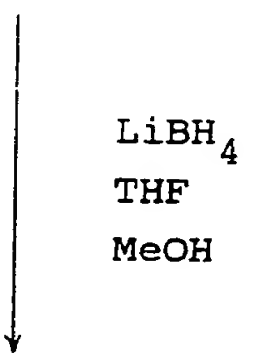
SCHEME 3

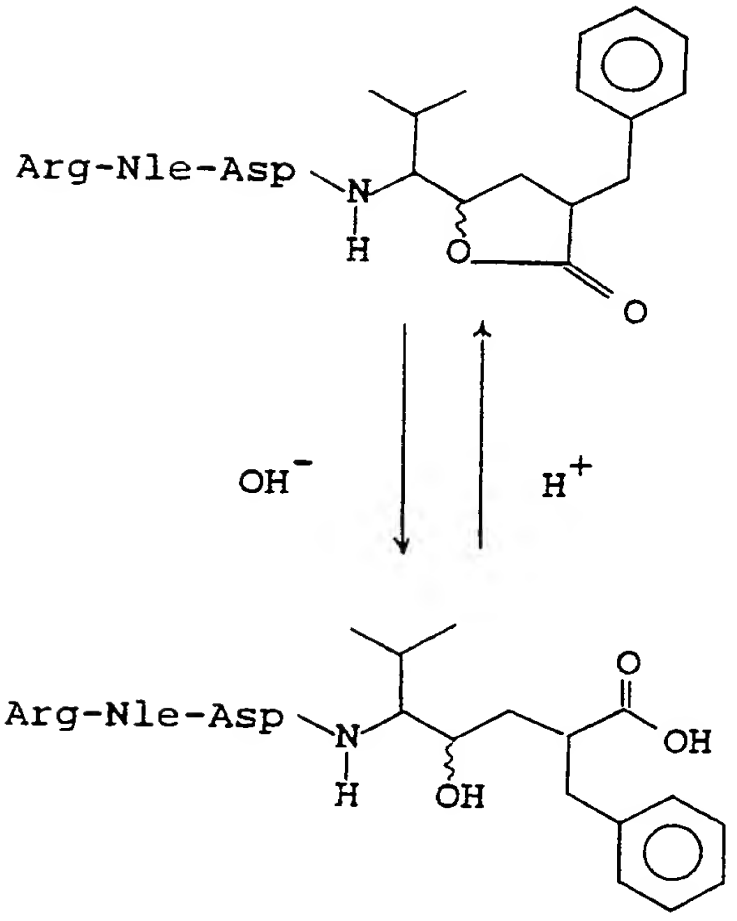


SCHEME 4



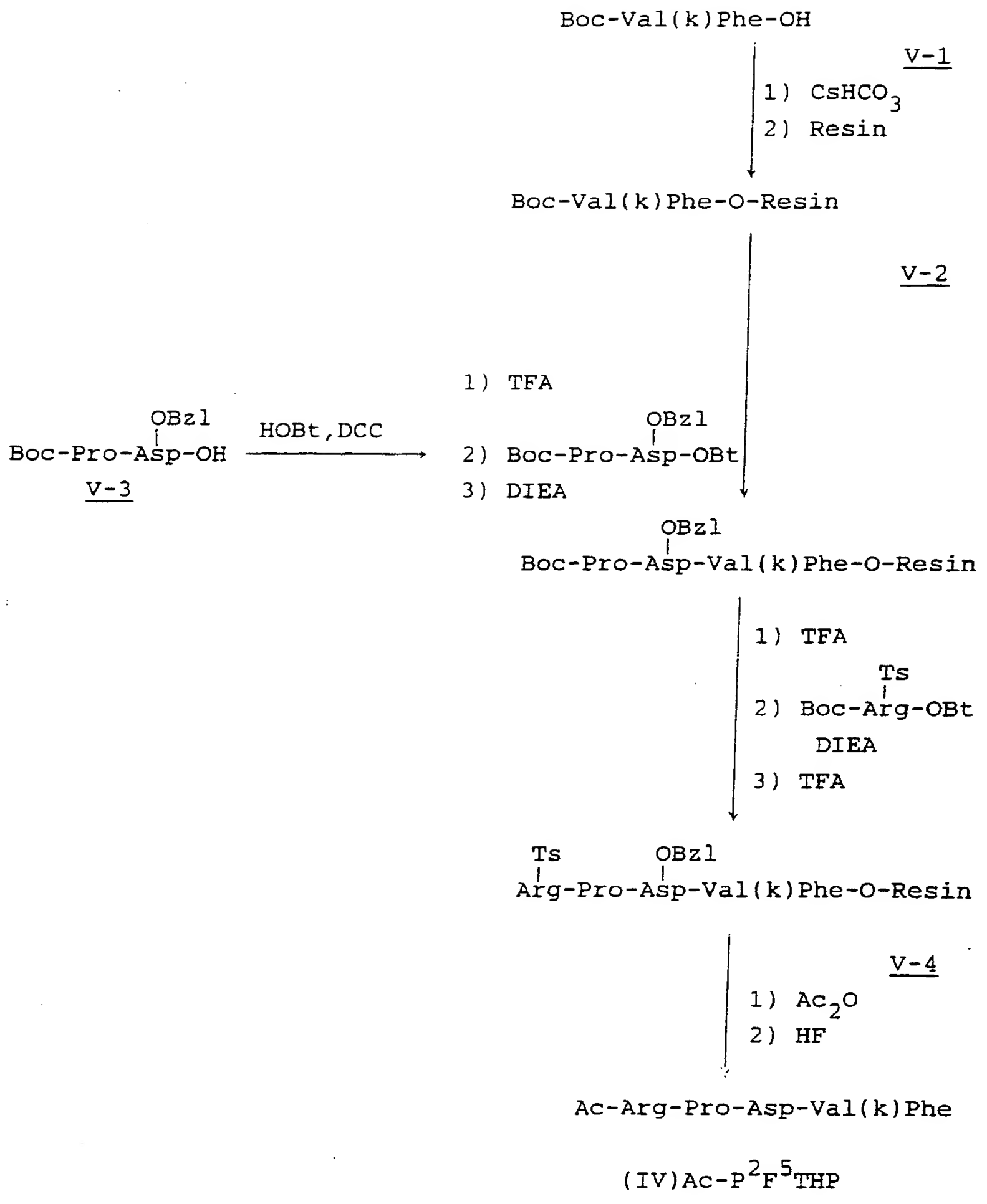
[Arg-Nle-Asp-Val(k)Phe]



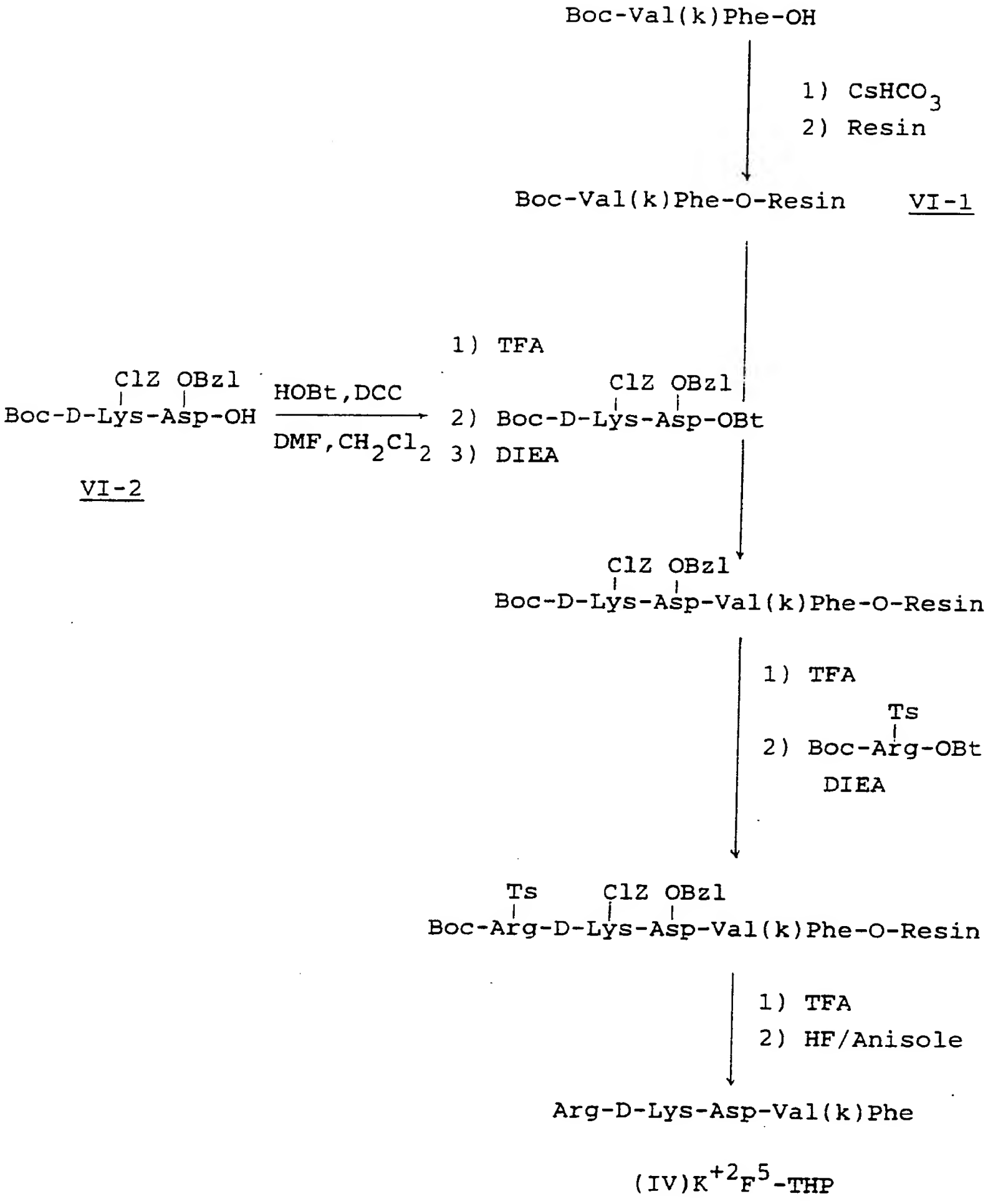


(IV)$Nle^{2}F^{5}$-THP($CHOHCH_2$)

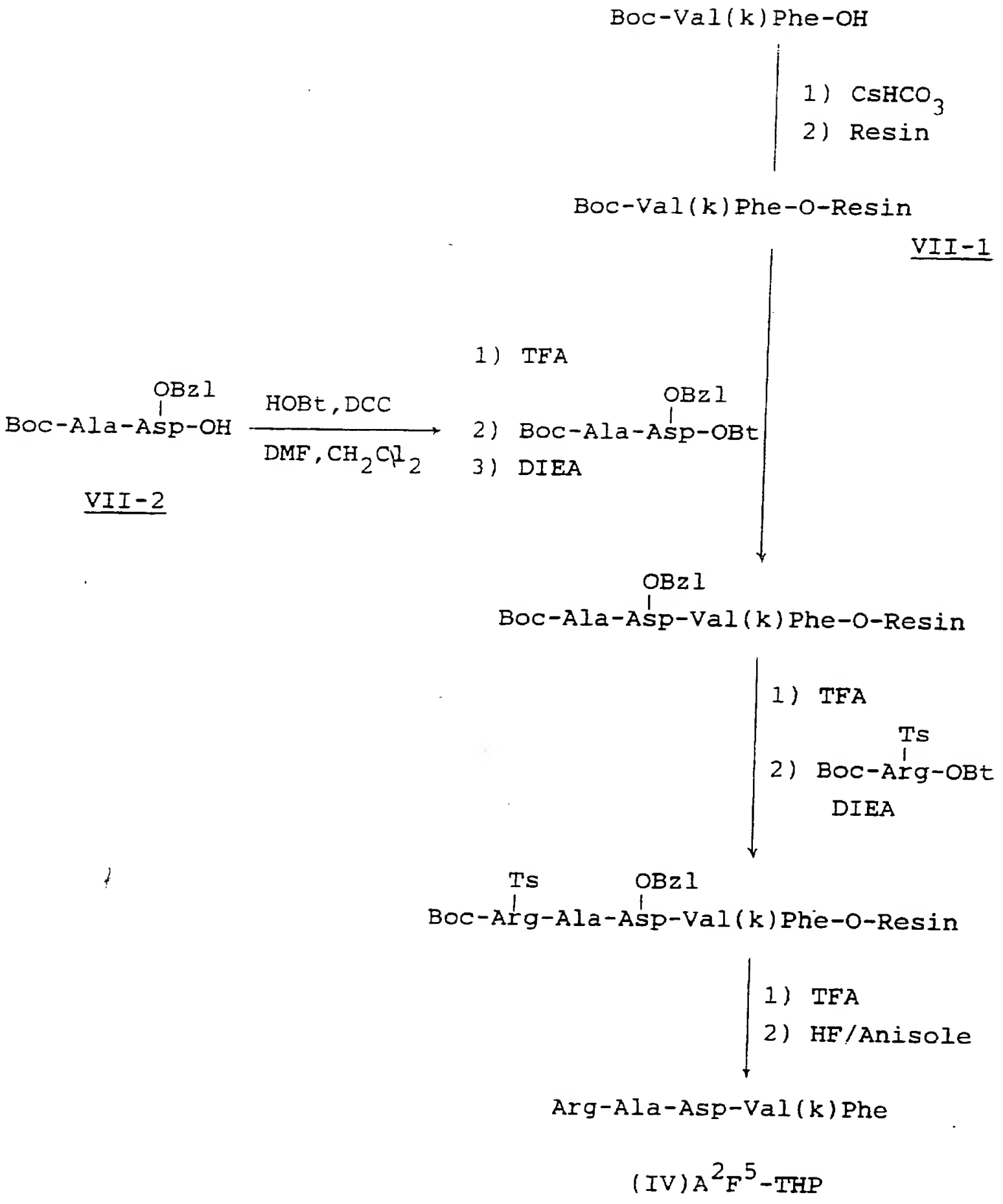
SCHEME 5



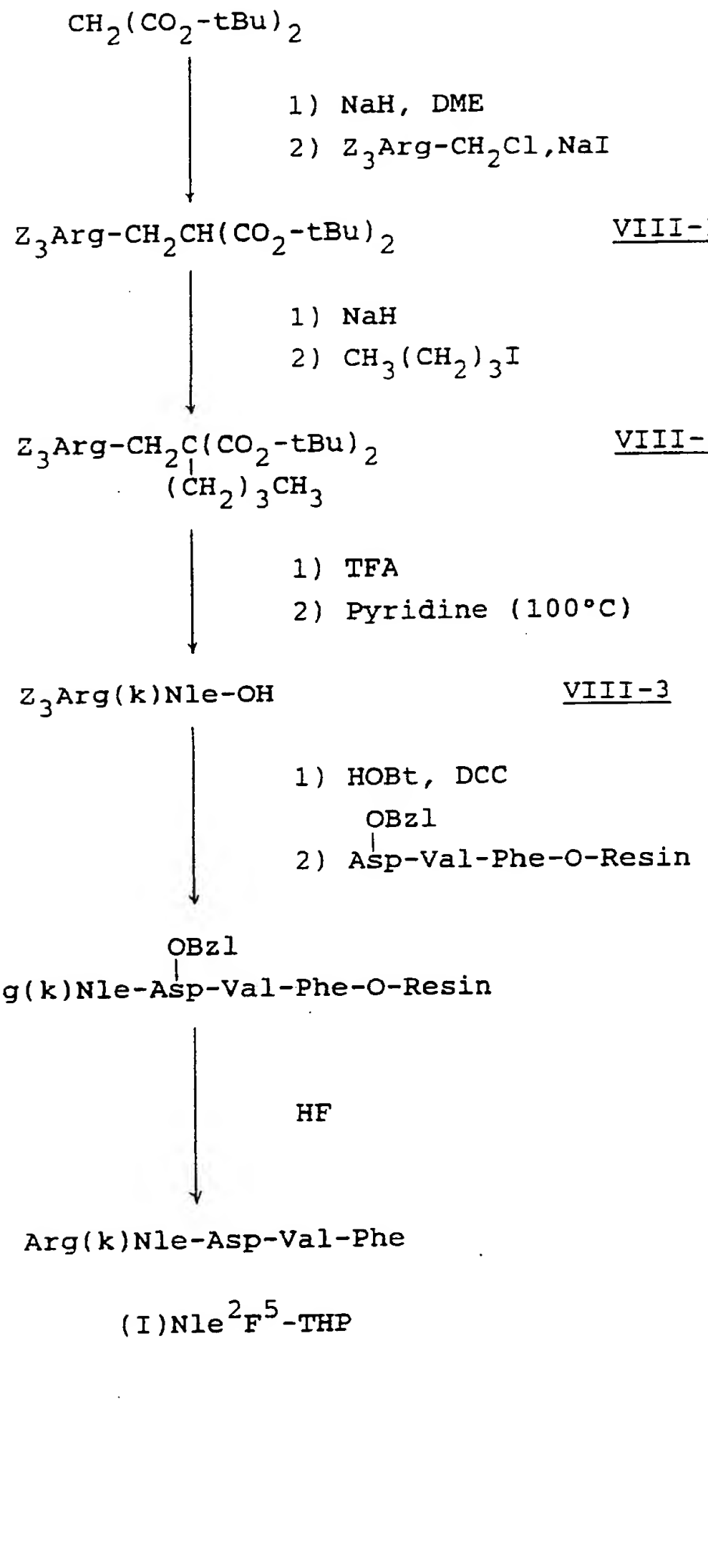
SCHEME 6



SCHEME 7



SCHEME 8



SCHEME 9

Z_3Arg(k)Nle-OH IX-1

↓ HOBt,BOP
DMF,NMM

Z_3Arg(k)Nle-OBt

↓ OBzl
H_2N-Asp-Val-Phe-MBHA

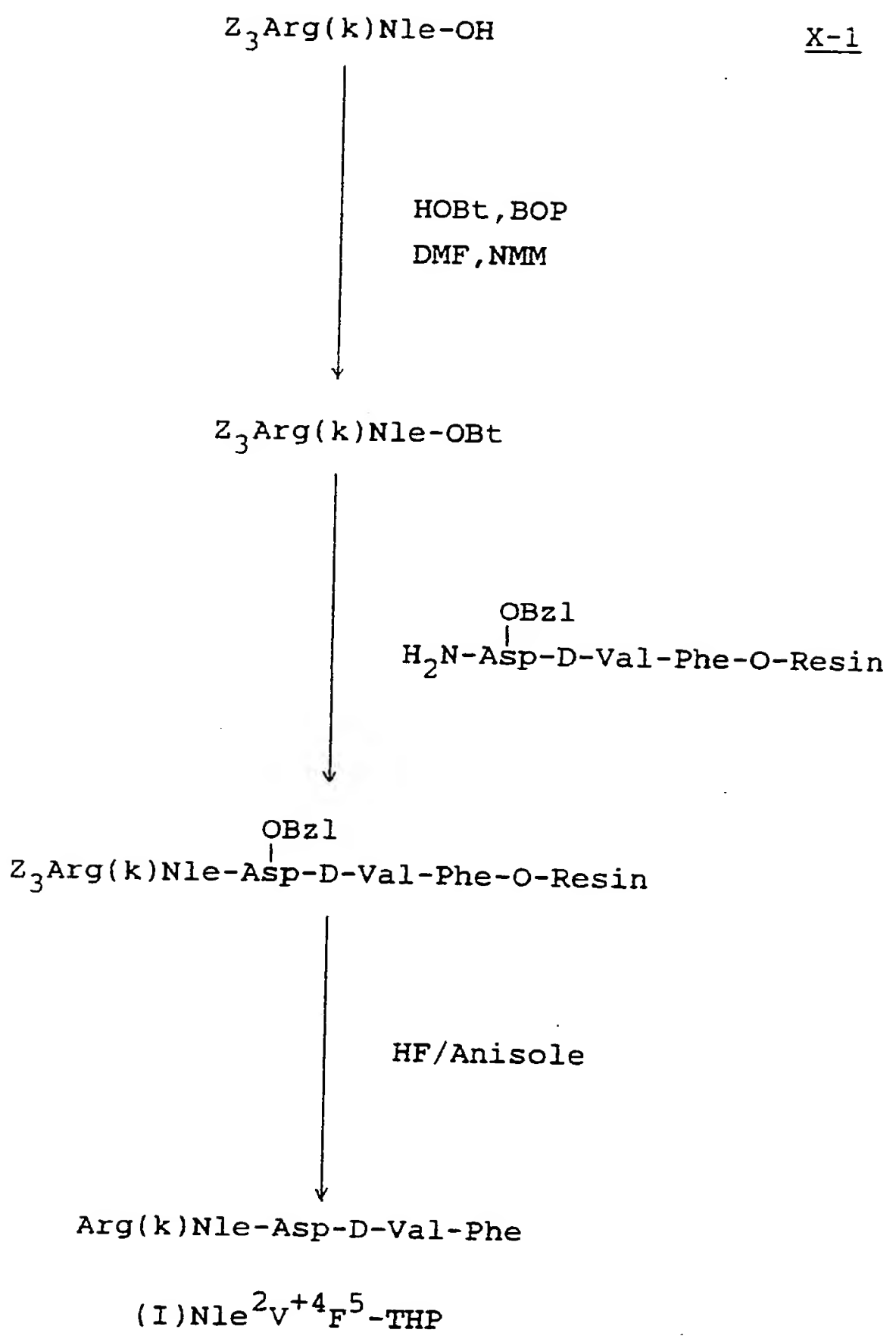
OBzl
Z_3Arg(k)Nle-Asp-Val-Phe-MBHA

↓ HF/Anisole

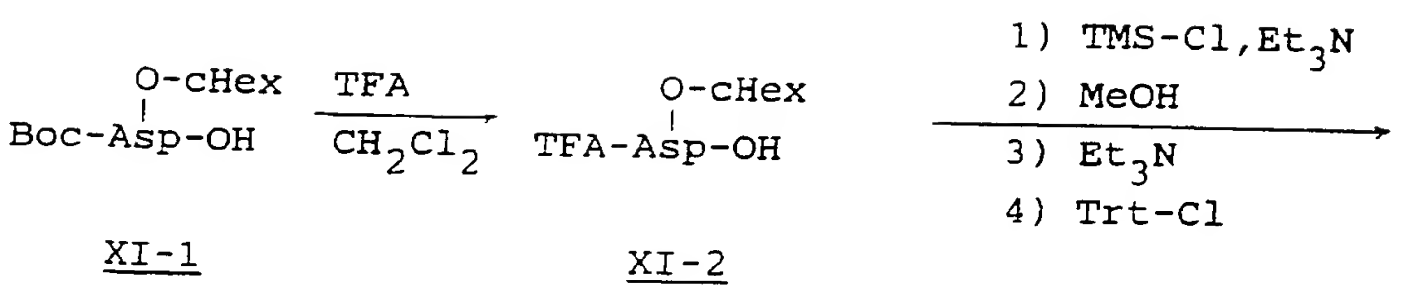
Arg(k)Nle-Asp-Val-Phe-NH_2

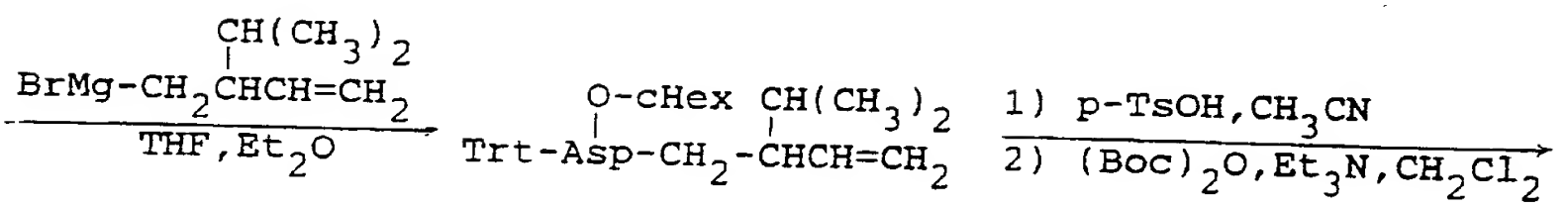
(I)Nle^2F^5-THP-NH_2

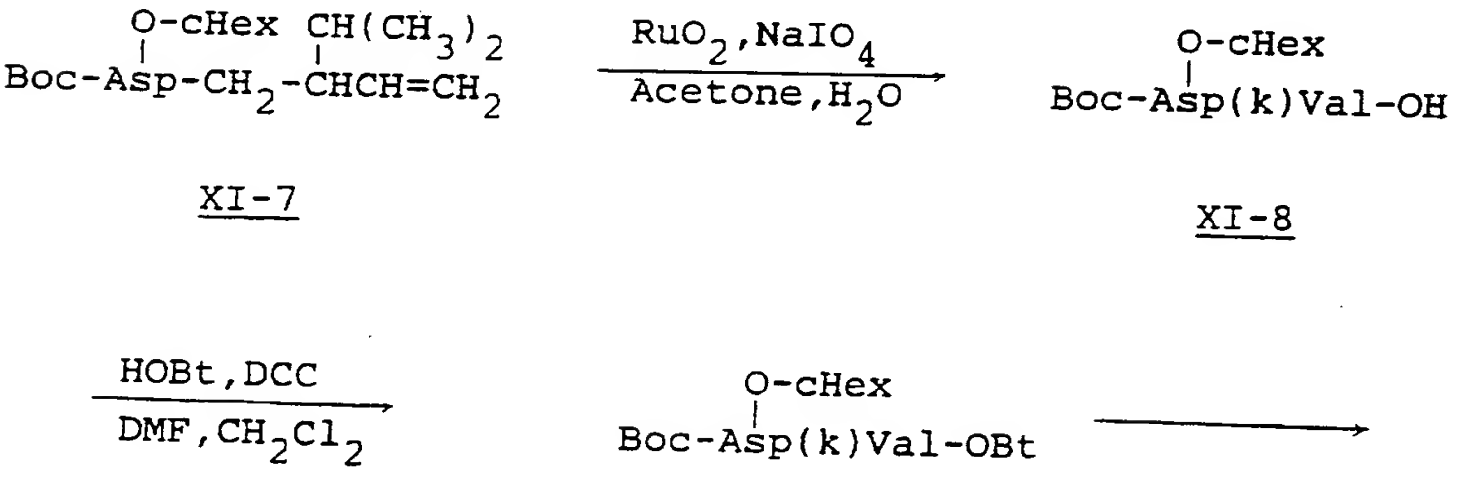
SCHEME 10



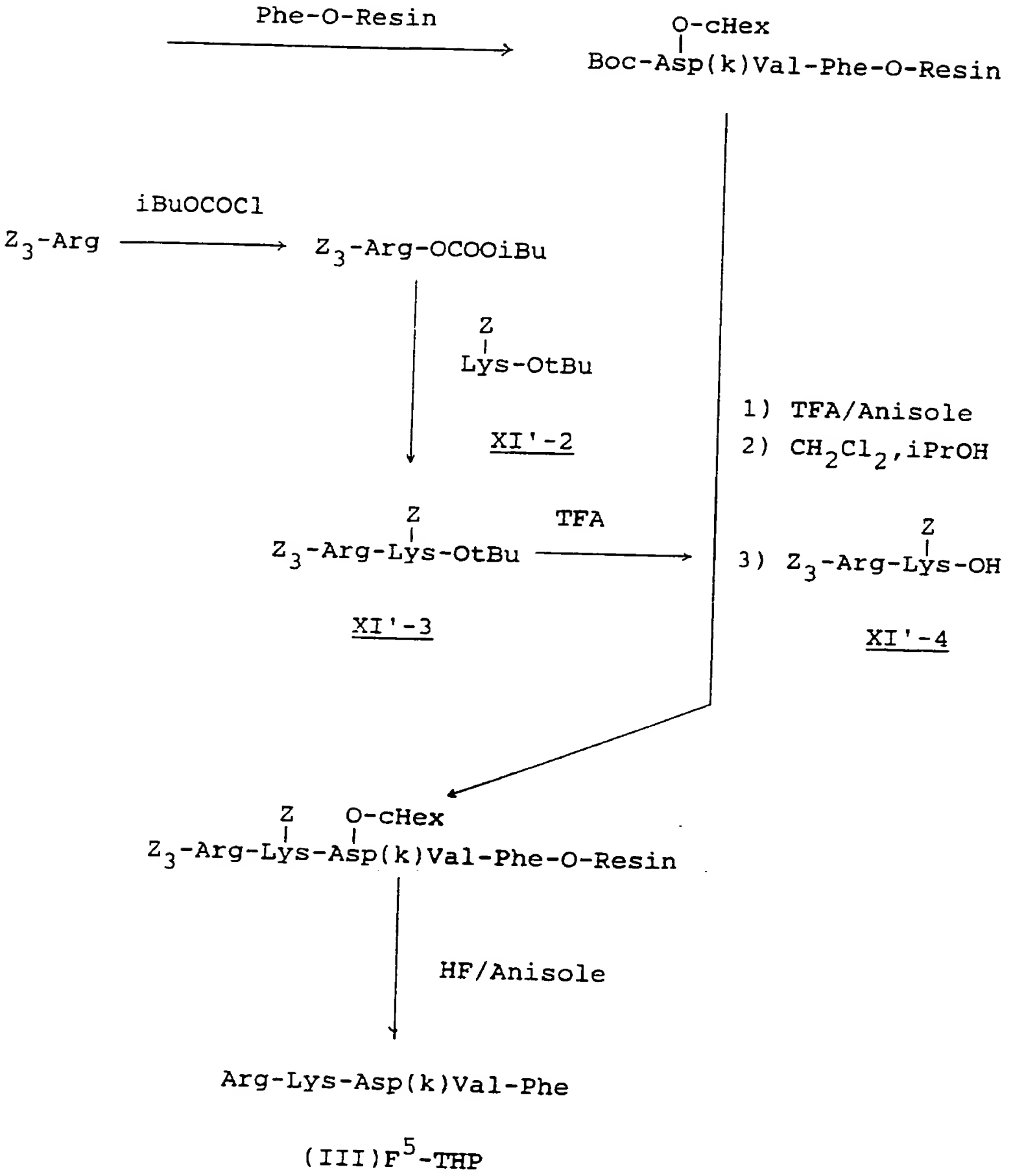
SCHEME 11



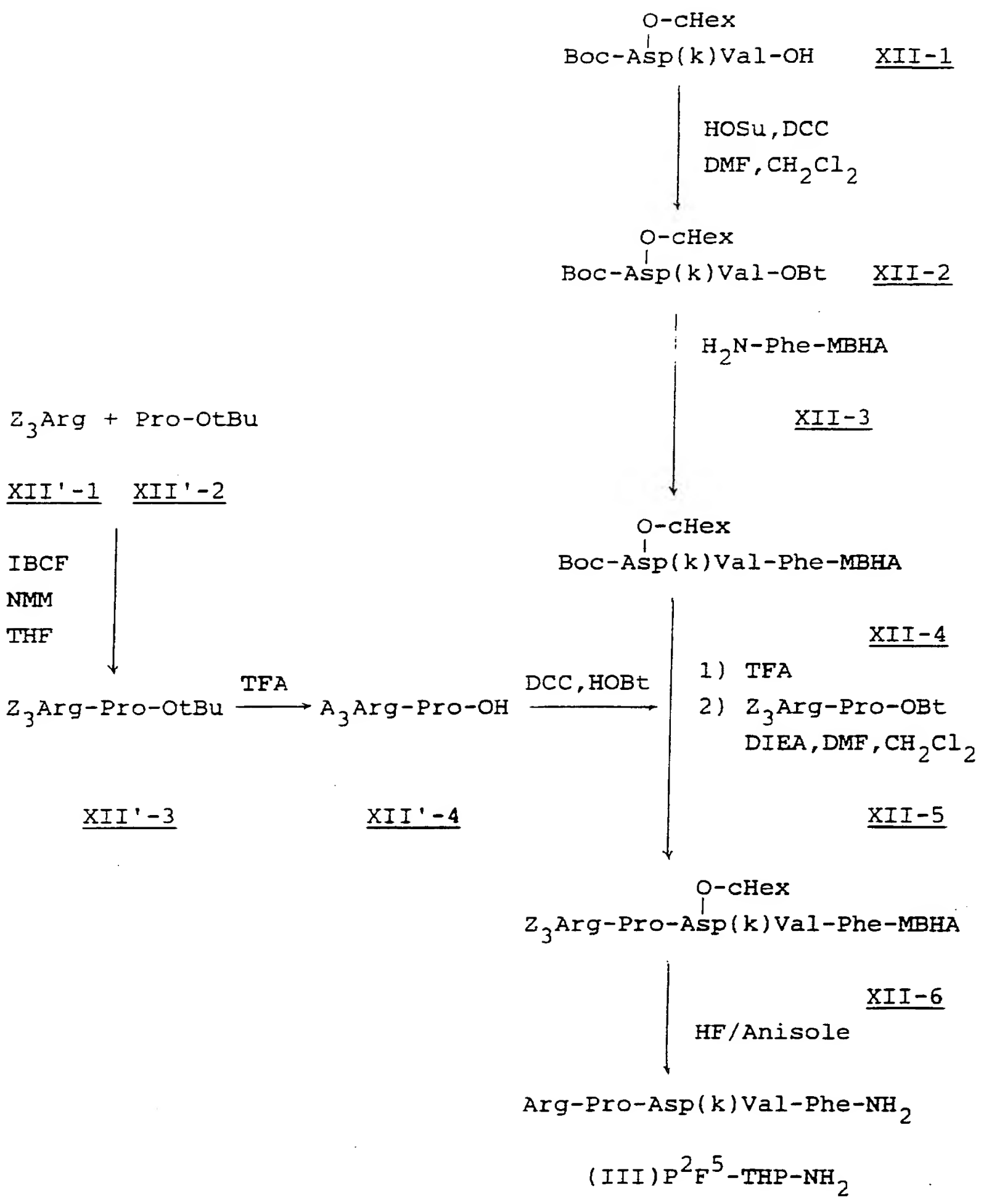




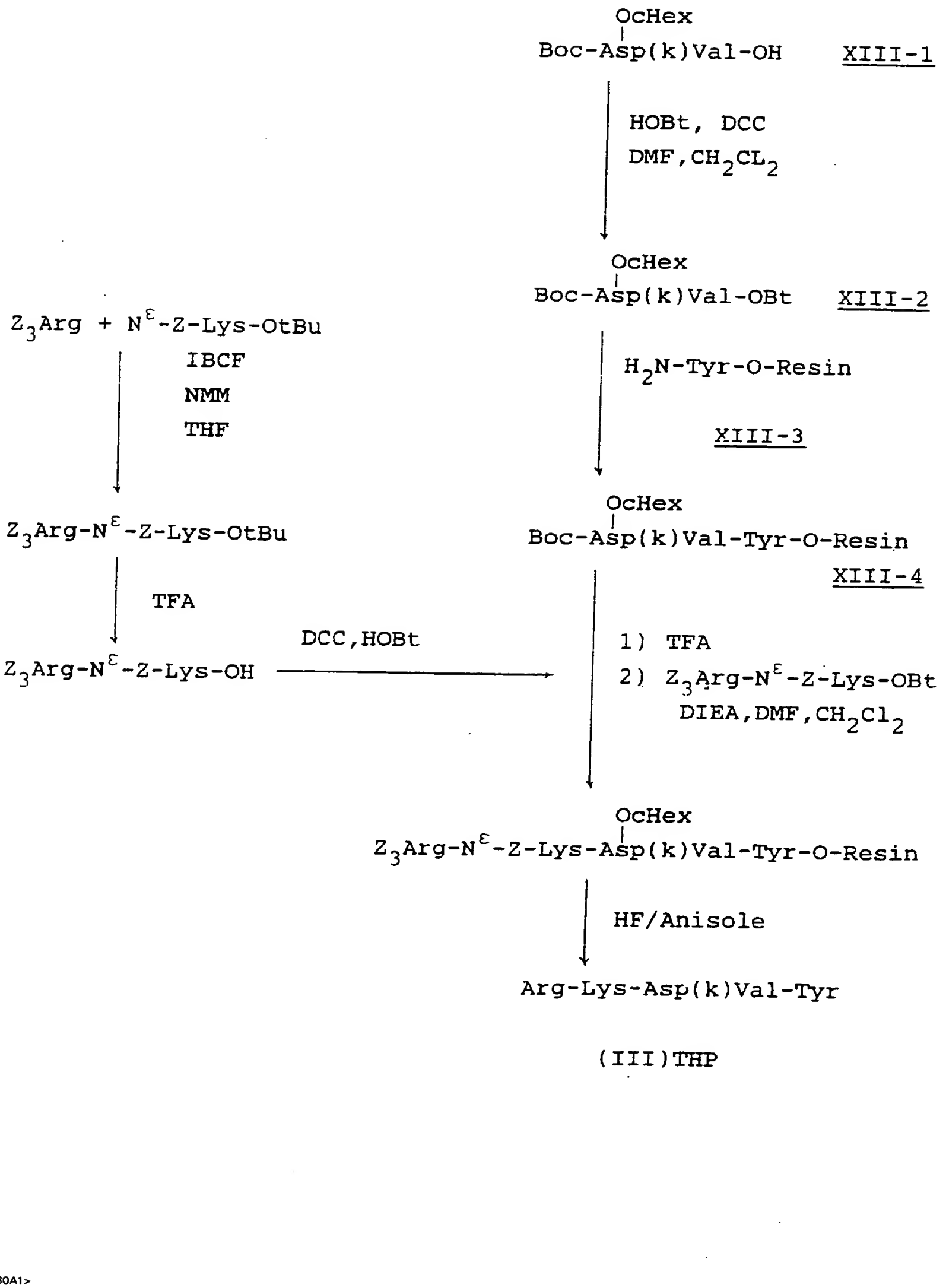
SCHEME 11 (Contd.)



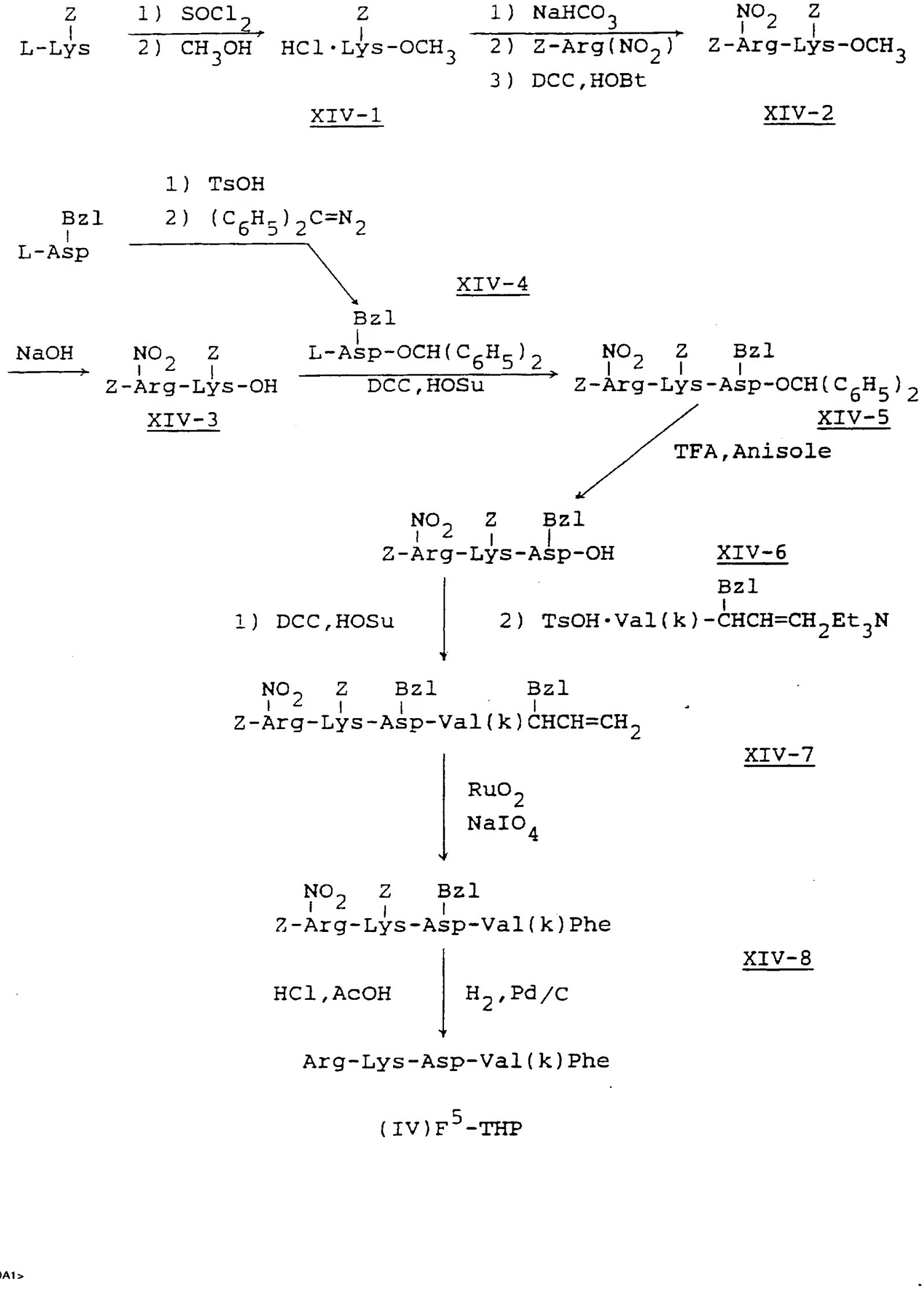
SCHEME 12



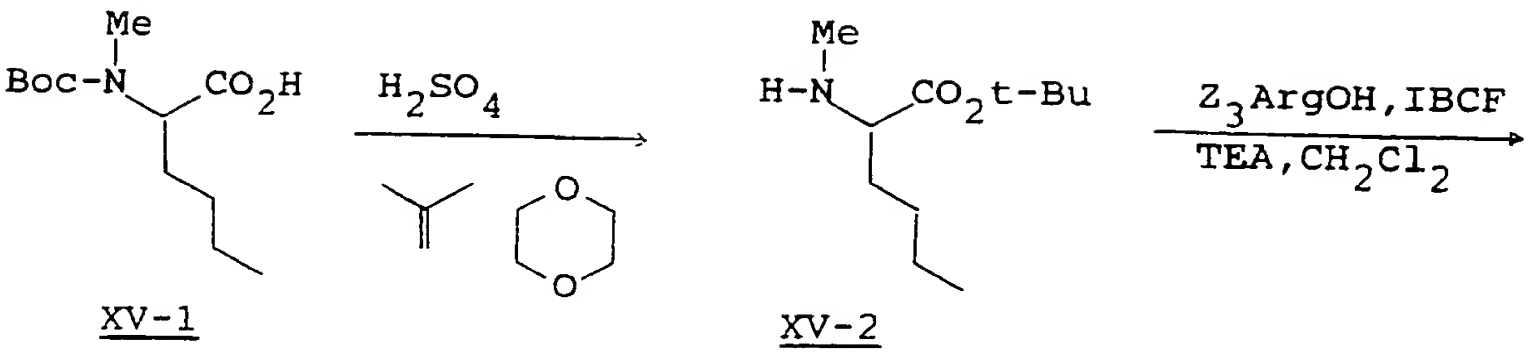
SCHEME 13



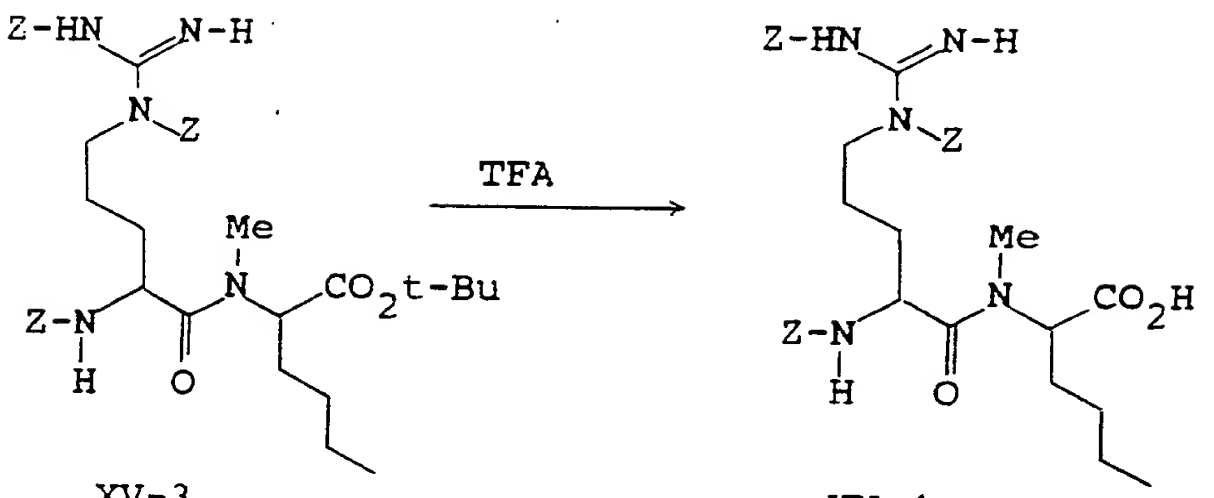
SCHEME 14



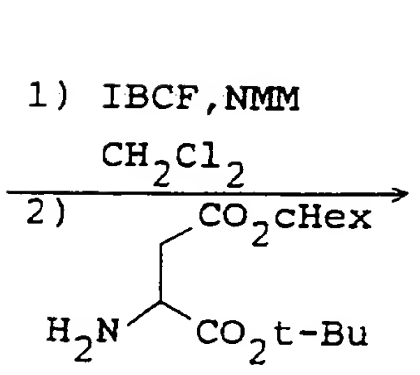
SCHEME 15



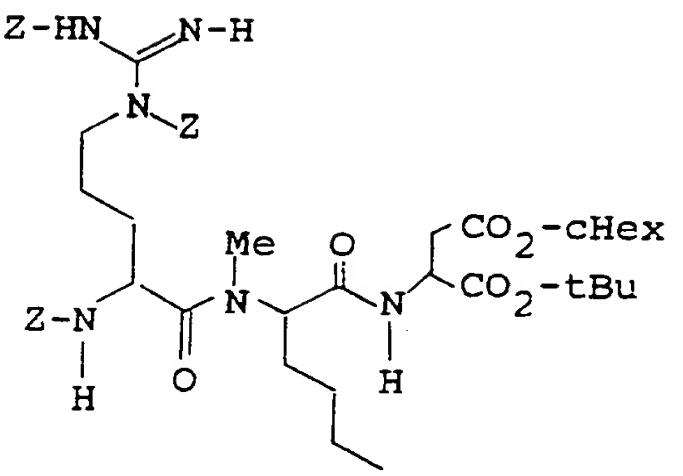
XV-1 XV-2



XV-3 XV-4

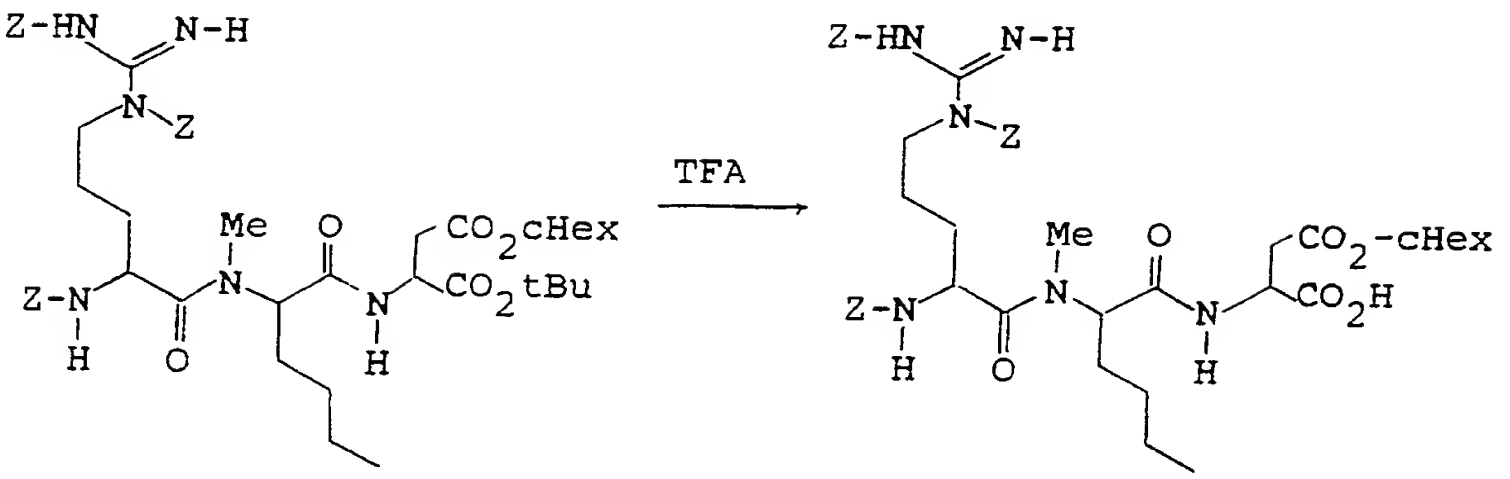


XV-5



XV-6

SCHEME 15 (continued)



XV-6 XV-7

1) HOBt,DCC
CH_2Cl_2 → Z_3-Arg-MeNle-Asp(OcHex)-Val(k)Phe-Resin
2) Val(k)Phe-Resin

XV-9

1) TFA,CH_2Cl_2
2) DIEA,CH_2Cl_2

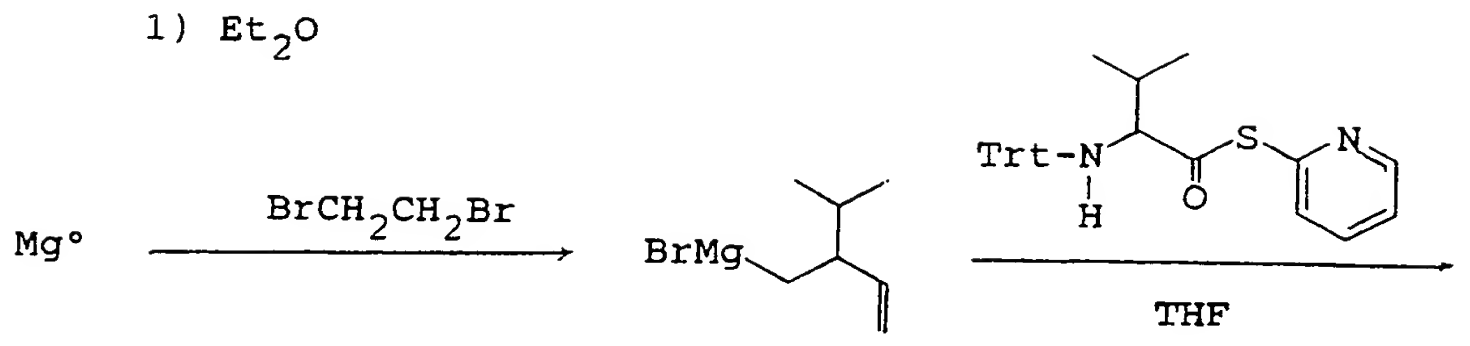
Boc-Val(k)Phe-Resin

XV-8

HF / anisole → HPLC / purification → Arg-MeNle-Asp-Val(k)Phe

(IV)N-MeNle^{2}F^{5}-THP

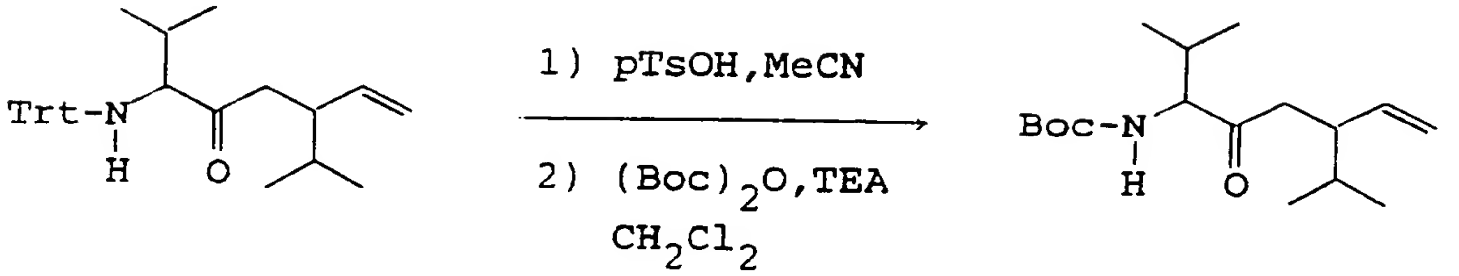
SCHEME 16



XVI-1

XVI-2

XVI-3

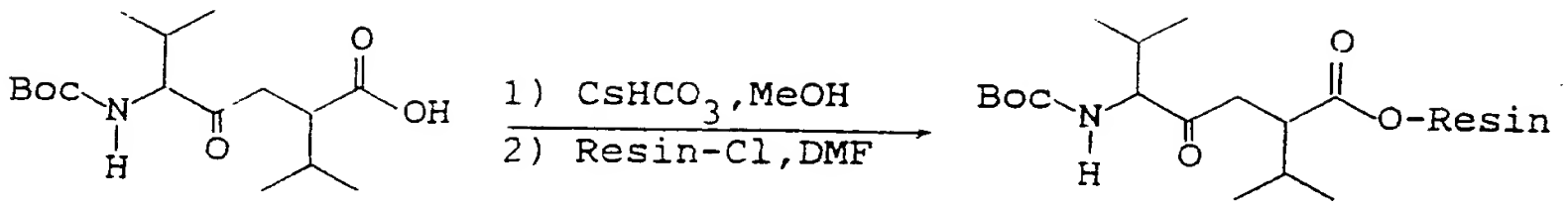


XVI-4

XVI-5

XVI-6

SCHEME 16 (Continued)



XVI-6 XVI-7

1)TFA,CH_2Cl_2
2)DIEA,CH_2Cl_2
3)XVI-9

Z_3-Arg-N^{α}-Me-Nle-Asp(OcHex)-Val(k)Val-OResin

XVI-10

DCC,HOBt
DMF,CH_2Cl_2

1) HF
2) HPLC

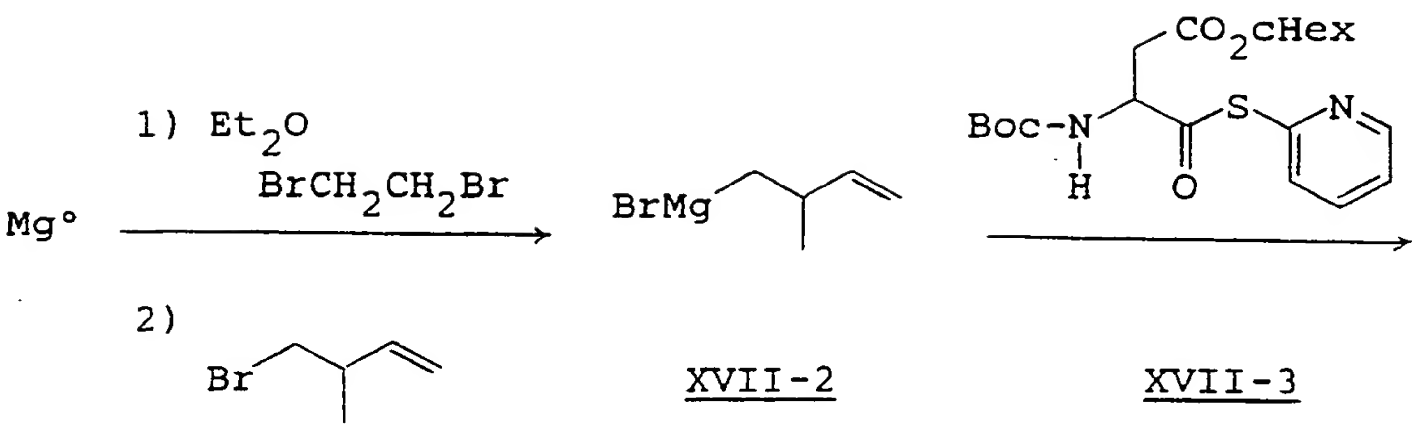
Z_3-Arg-N^{α}-Me-Nle-Asp(OcHex)OH

XVI-8

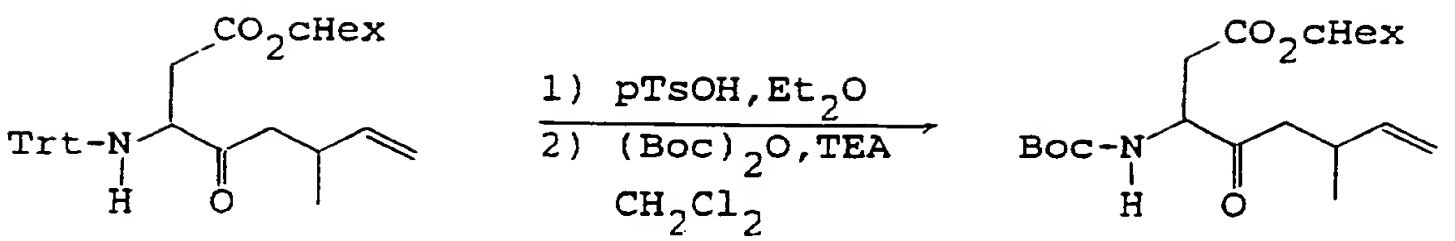
Arg-NMe-Nle-Asp-Val(k)Val

(IV)N-MeNle^2V^5-THP

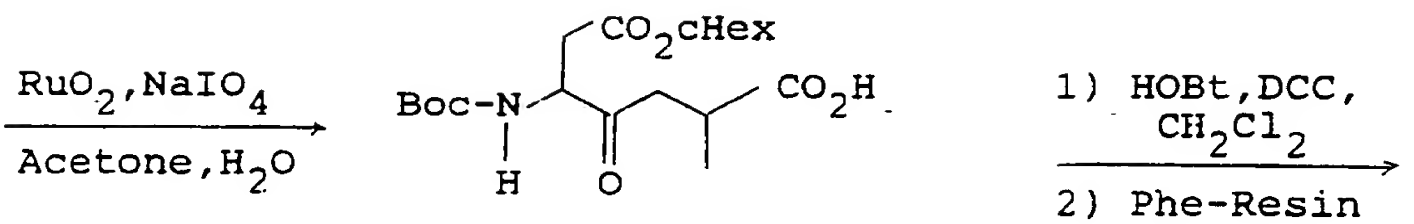
SCHEME 17



XVII-1 XVII-2 XVII-3

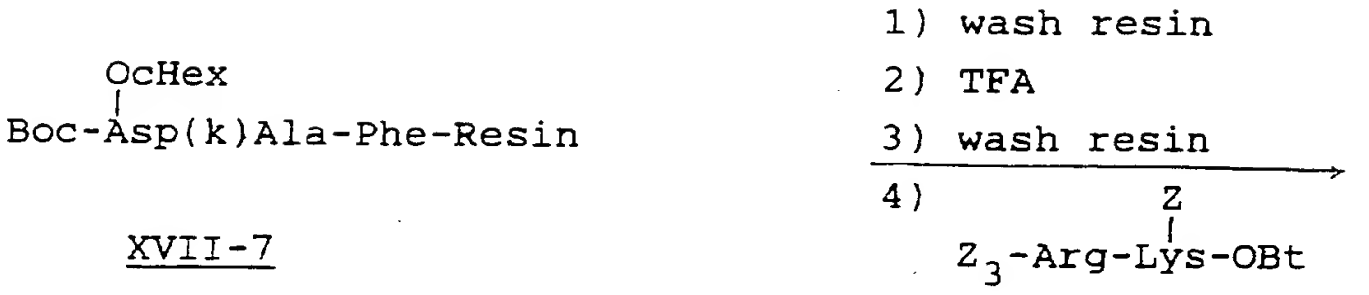


XVII-4 XVII-5

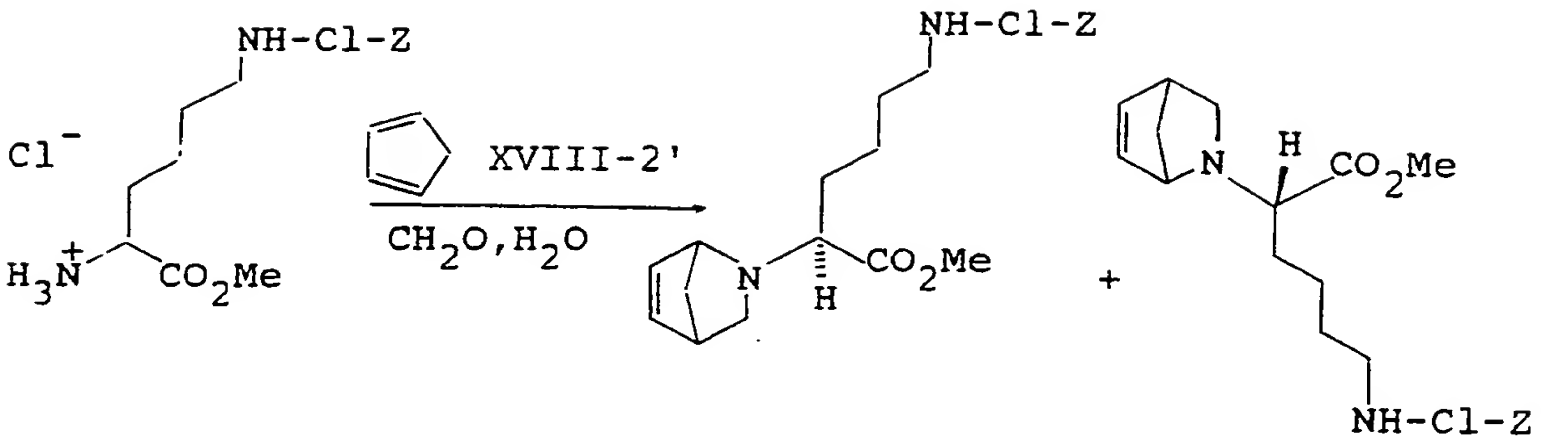


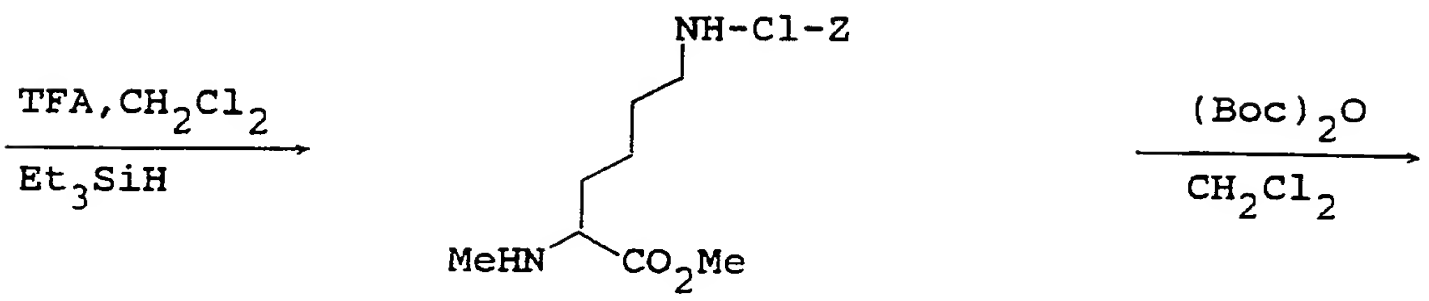
XVII-6

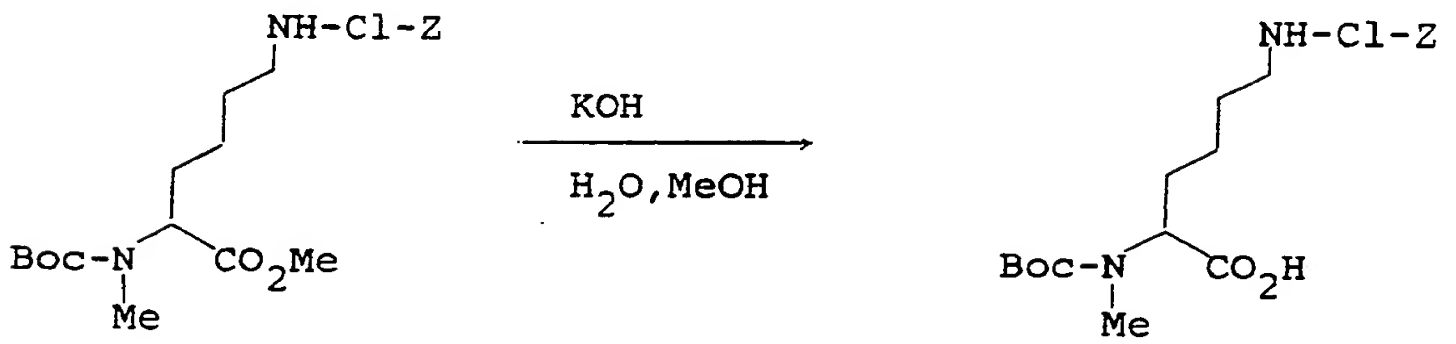
SCHEME 17 (Continued)



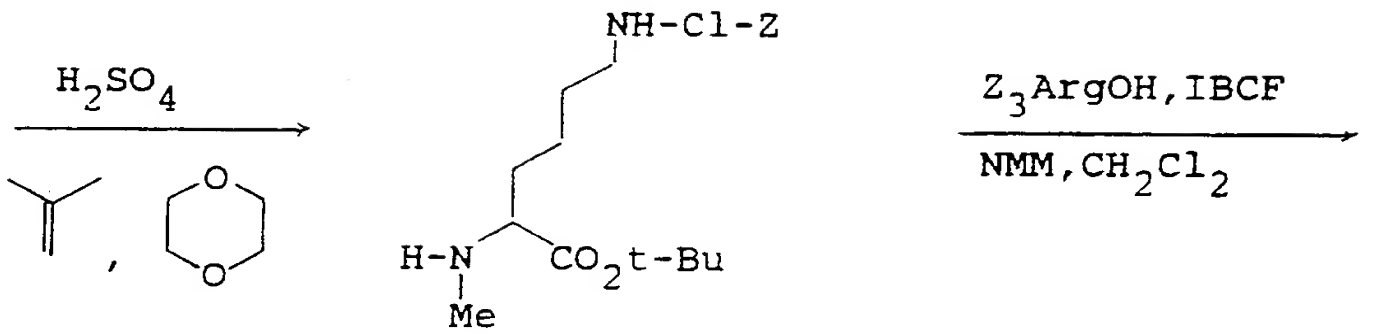
SCHEME 18



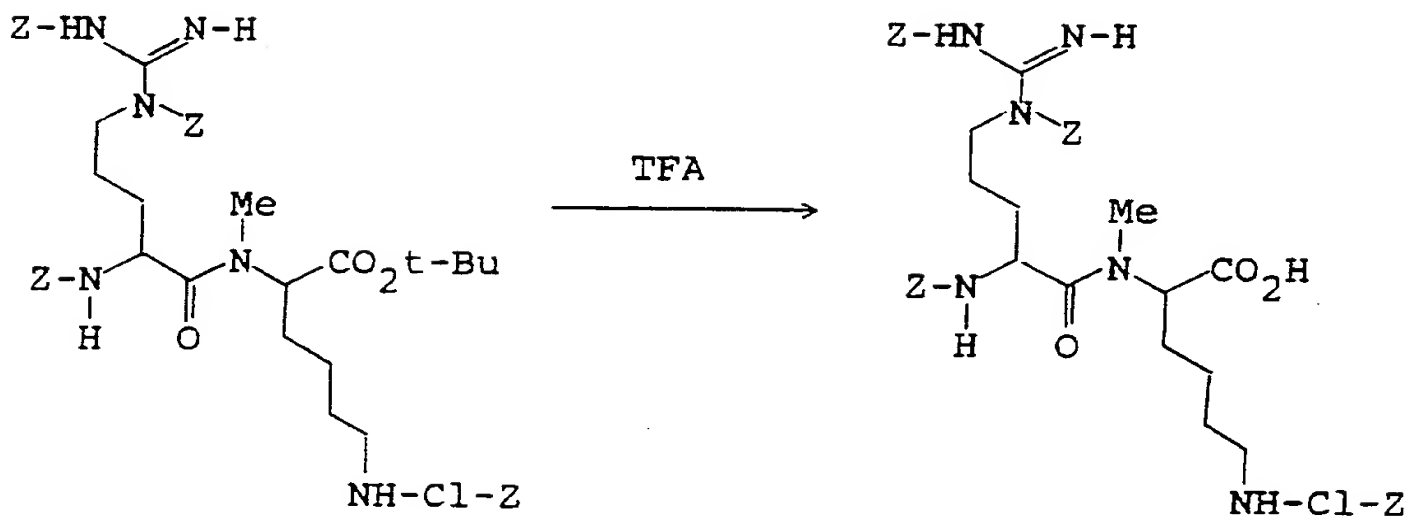




SCHEME 18 (Continued)

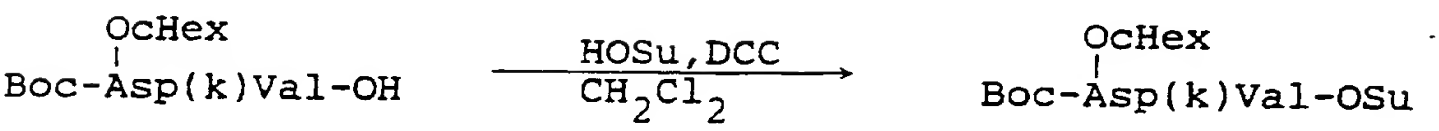


XVIII-8'



XVIII-9'

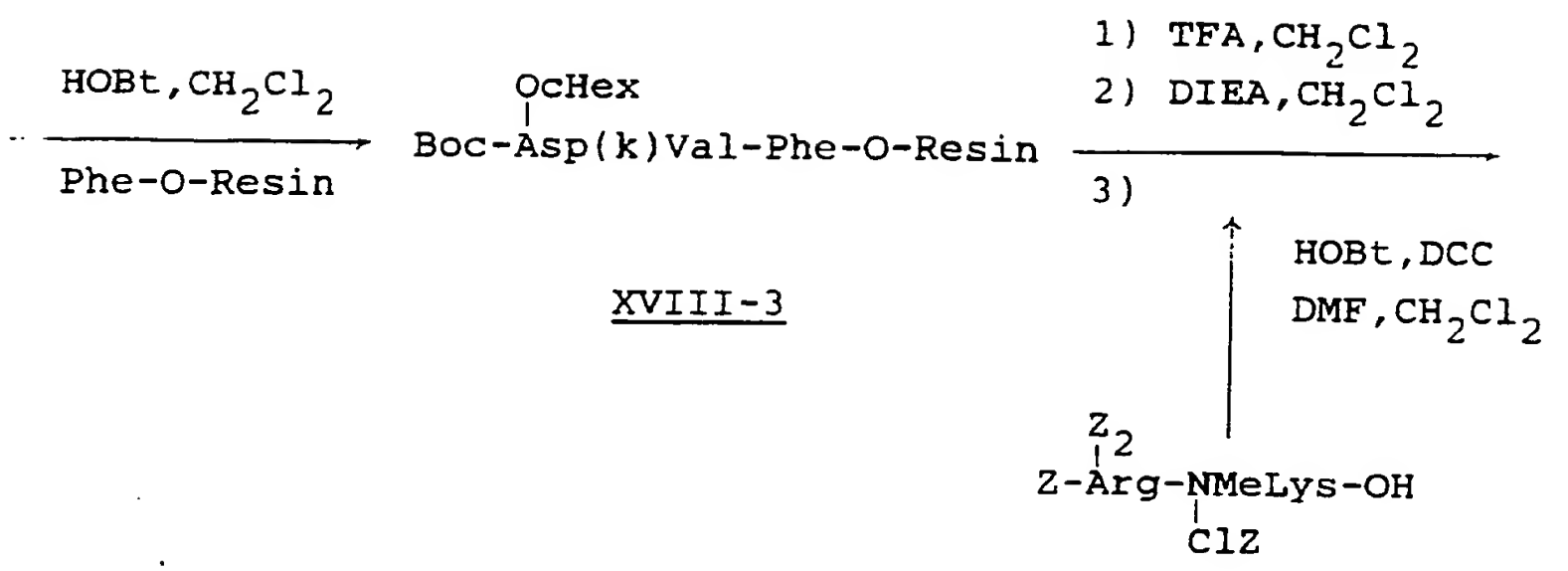
XVIII-10'

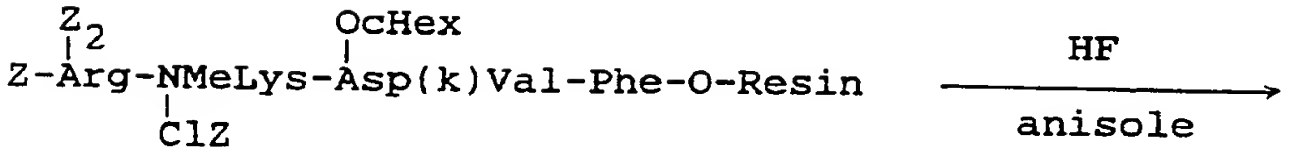


XVIII-1

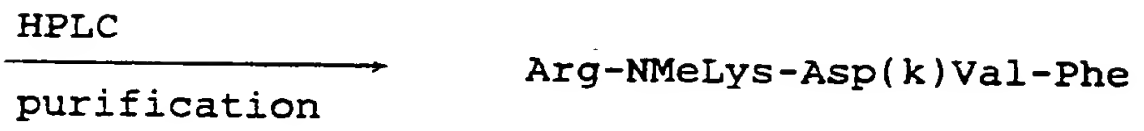
XVIII-2

SCHEME 18 (Continued)



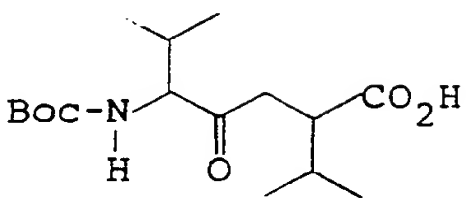


XVIII-4



(III)N-MeK^2F^5-THP

SCHEME 19



XIX-1

1) $CsHCO_3$
2) Merrifield resin

Boc-Val(k)Val-Resin

XIX-2

1) wash resin
2) TFA
3) wash resin
4) Boc-Nle-Asp(OBzl)-OBt

Boc-Nle-Asp(OBzl)-Val(k)Val-Resin

XIX-4

XIX-3

1) wash resin
2) TFA
3) wash resin
4) Boc-Arg(Ts)-OBt

Boc-Arg(Ts)-Nle-Asp(OBzl)-Val(k)Val-Resin

XIX-6

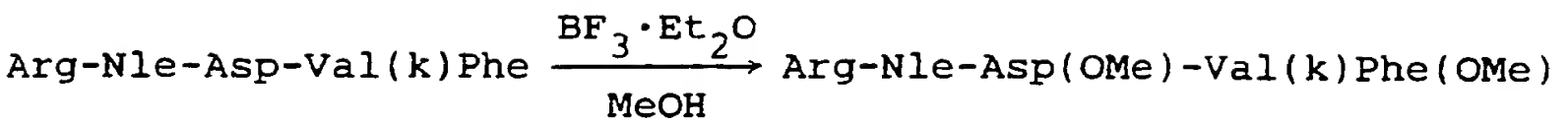
XIX-5

1) HF cleavage
2) HPLC purification

Arg-Nle-Asp-Val(k)Val

(IV)Nle^2V^5-THP

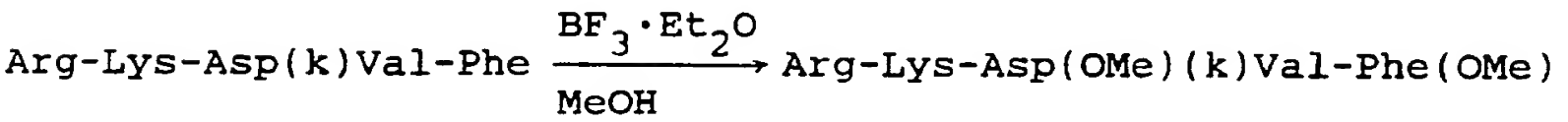
SCHEME 20



(IV)Nle^2F^5-THP
(in Example 3)

(IV)$Nle^2D^3(OMe)F^5(OMe)$-THP

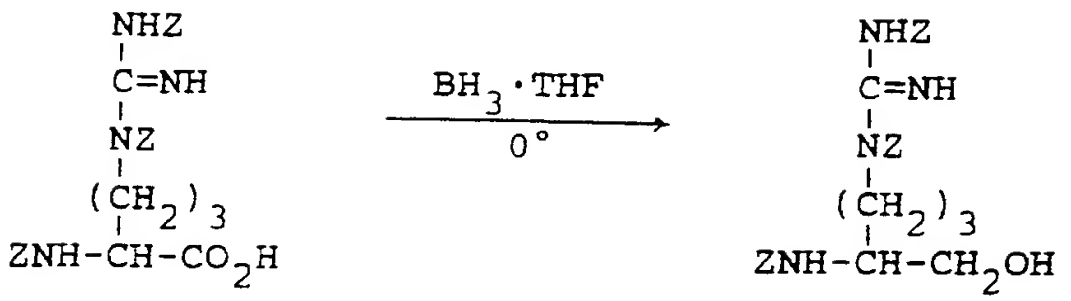
SCHEME 21



(III)F^5-THP
(in Example 11)

(III)$D^3(OMe)F^5(OMe)$-THP

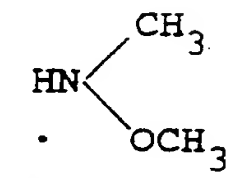
SCHEME 22



Method A (79%)

BOP

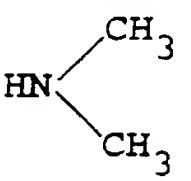
TEA



Method B (95%)

1. iBuOCOCl

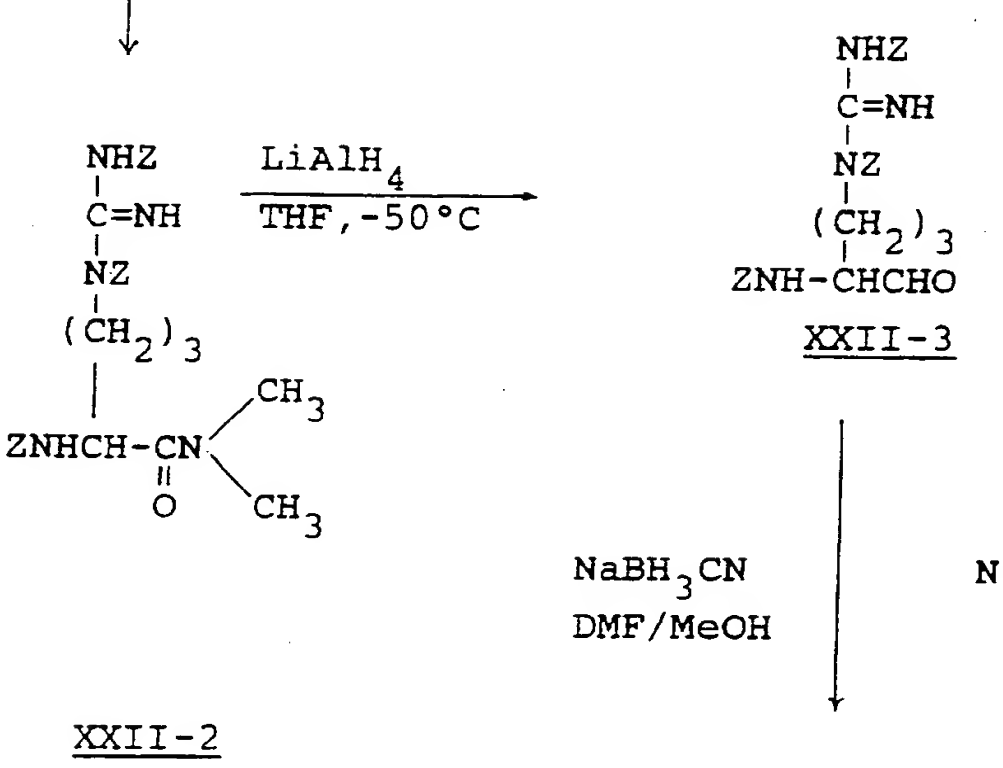
NMM



XXII-1

SO_3·pyridine

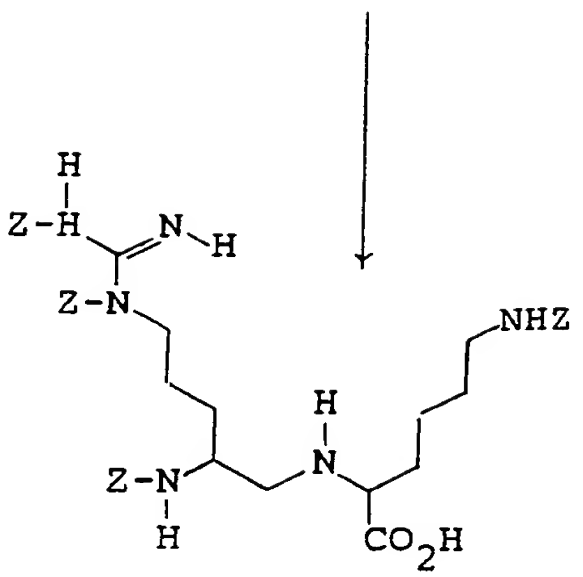
DMSO



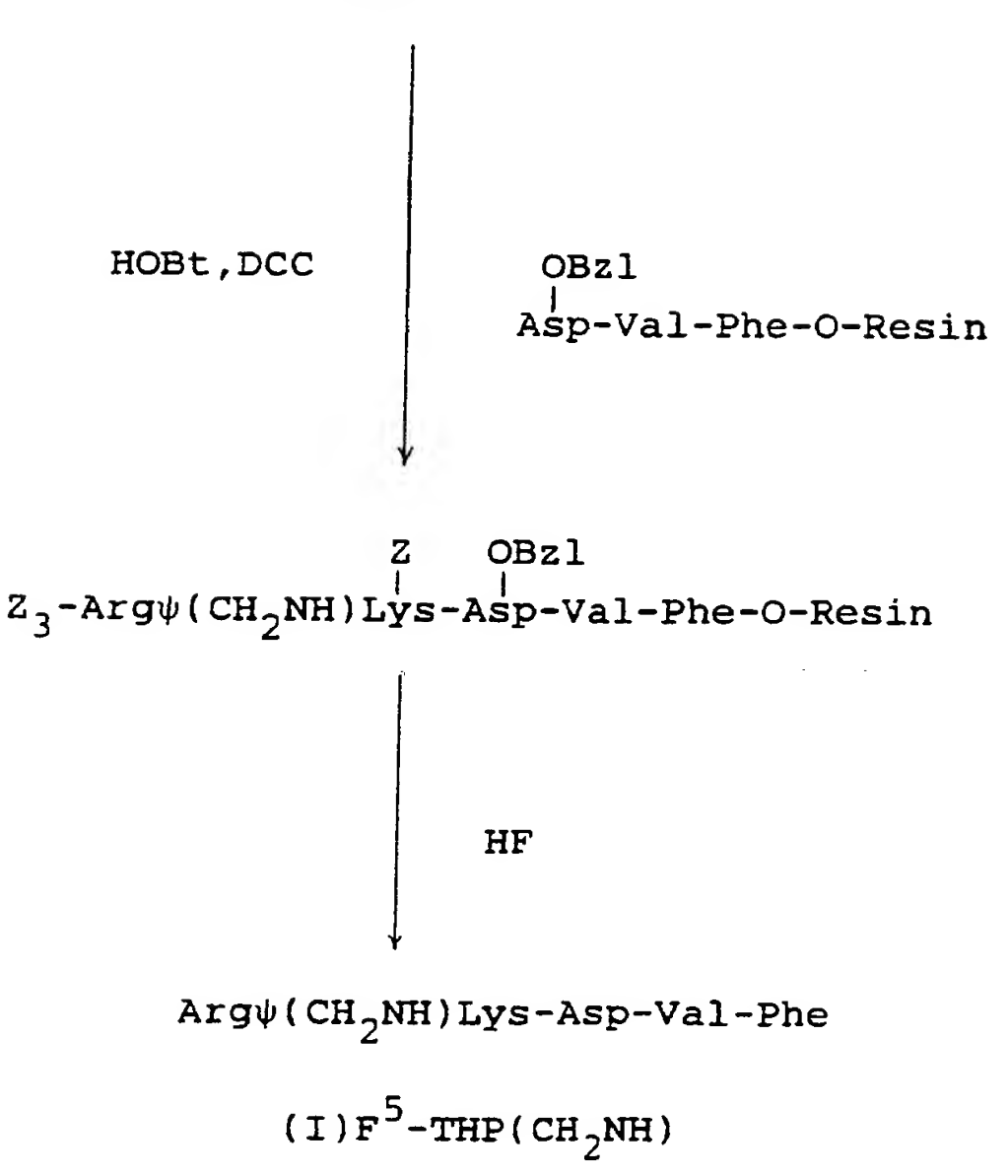
XXII-3

XXII-2

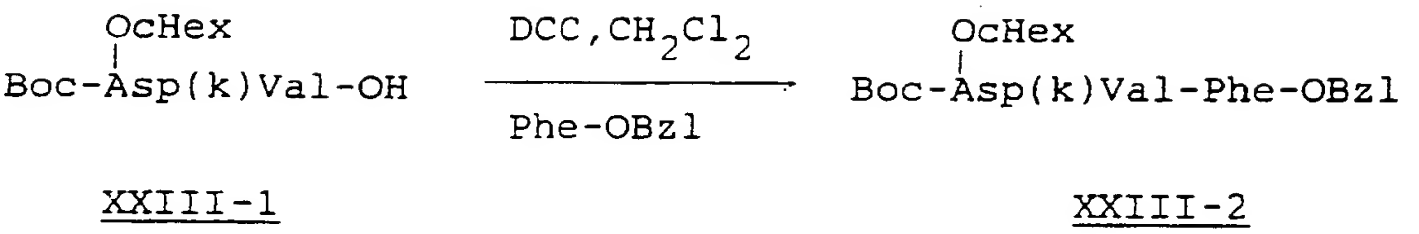
SCHEME 22 (Continued)



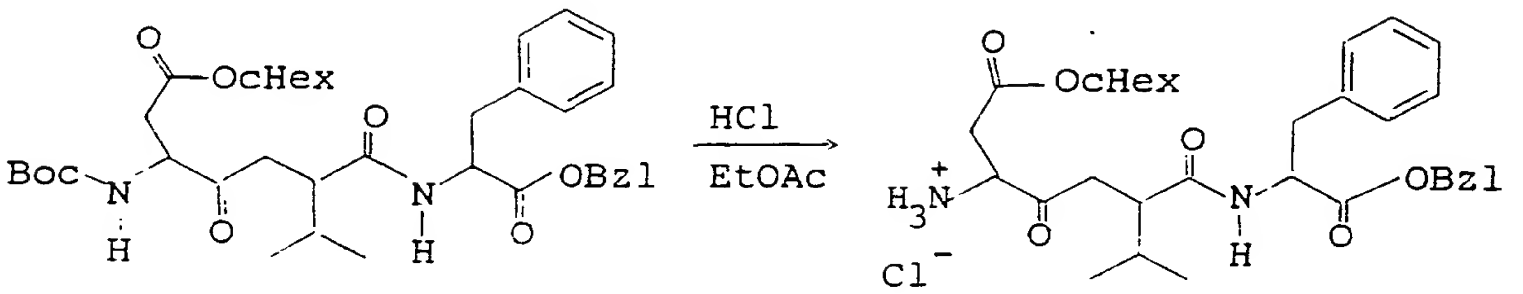
XXII-4



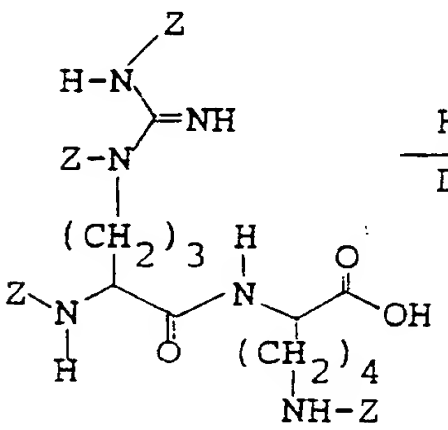
SCHEME 23



XXIII-1 XXIII-2



XXIII-3 XXIII-4

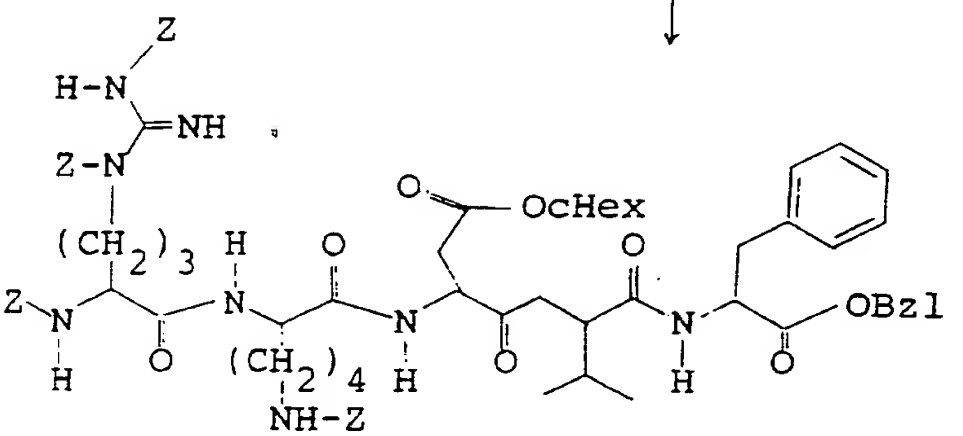


XXIII-5

HOBT, DCC
DMF, CH_2Cl_2

1) XXIII-6
2) DIEA

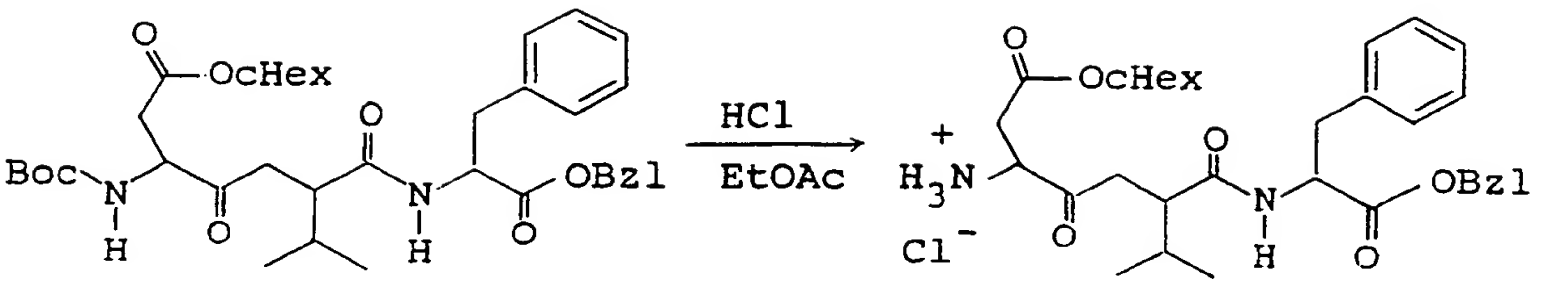
1) HF
2) HPLC



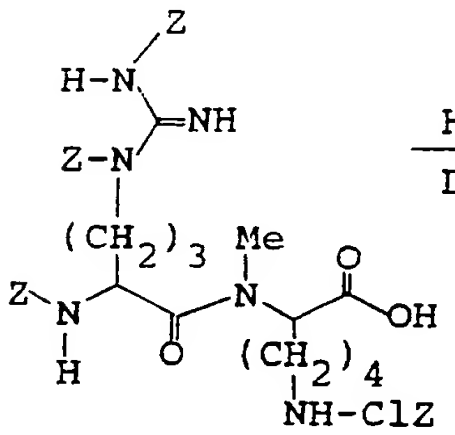
XXIII-7

Arg-Lys-Asp(k)Val-Phe
(III)F^5-THP

SCHEME 24



XXIV-1 XXIV-2

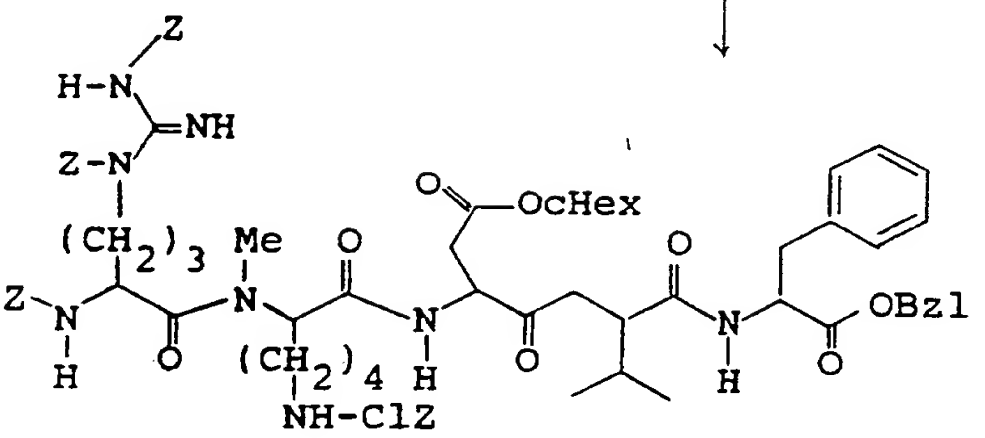


HOBT,DCC
DMF,CH_2Cl_2

1) XXIV-4
2) DIEA

XXIV-3

1) HF
2) HPLC



XXIV-5

Arg-NMeLys-Asp(k)Val-Phe
(III)N-MeK^2F^5-THP

In the examples, the following usual abbreviations within the peptide chemistry are used:

TMS-Cl	chlorotrimethyl silane
Trt-Cl	triphenylmethyl chloride
-OBt	O-benzotriazole
DCC	dicyclohexylcarbodiimide
THF	tetrahydrofuran
Boc	tert-butyloxycarbonyl
TsOH	p-toluenesulfonic acid (Tosyl)
-OBzl	benzyloxy group
IpOH	isopropanol
-ClZ	chlorobenzyloxycarbonyl group
-cHex	cyclohexyl group
TEA	triethylamine
$(Boc)_2O$	di-tert-butyl dicarbonate
Ac_2O	acetic anhydride
DMF	N,N-dimethylformamide
DIEA	diisopropylethylamine
Z	benzyloxycarbonyl group
IBCF	isobutylchloroformate
NMM	N-methylmorpholine
TFA	trifluoroacetic acid
DME	dimethoxy ethane
MBHA	p-methylbenzhydryl amine resin
BOP	benzotriazol-1-yloxytris (dimethylamino)phosphonium hexafluorophosphate

DCU	Dicyclohexyl urea
HOSu	N-hydroxysuccinimide
HF	hydrofluoric acid
RuO_2	ruthenium dioxide
$CsHCO_3$	cesium bicarbonate
Et_3SiH	triethyl silane
BF_3	boron trifluoride
BH_3	boron hydride (borane)
$LiAlH_4$	lithium aluminum hydride
$NaBH_3CN$	sodium cyanoborohydride

The following additional abbreviations are also used:

RT	retention time
TLC	thin layer chromatography
HPLC	high performance liquid chromatography

EXAMPLES

Example 1

Preparation of Arg-Lys-Asp-Val(k)Phe ((IV) F^5-THP)

N-Trityl-L-valine-mercaptopyridine ester (I-1)

25.0 g (0.21 moles) L-valine (Sigma) and 27.1 mL (23.2 g, 0.21 moles) chlorotrimethylsilane (Aldrich) in 500 mL chloroform/acetonitrile (5/1) was stirred at reflux under argon for 2 hours. The mixture was then cooled to 0°C and treated, dropwise, with 59.3 mL (43.0 g, 0.43 moles) triethylamine then, dropwise, with a solution of 59.4 g (0.21 moles) triphenylmethyl chloride (Aldrich) in 200 mL chloroform. The mixture was stirred at room

temperature for 1.5 hours then partitioned between 750 mL 5% citric acid and 750 mL diethyl ether. The organic layer was washed with 1 molar sodium hydroxide (2×500 mL) then water (500 mL). The aqueous washed were combined, cooled to 0°C, and neutralized (pH=6-7) with glacial acetic acid. The mixture was extracted with ether (3×500 mL). The organic extracts were combined, dried on sodium sulfate and concentrated. The white foam (27.3 g) was dissolved in 300 mL ethyl acetate and treated with 8.5 g (76.4 mmol) 2-mercaptopyridine. The solution was cooled to 0°C and treated with 15.75 g (76.4 mmol) dicyclohexyl-carbodiimide. The solution was stirred at 0°C for 3 hours then at room temperature for 16 hours. The mixture was filtered and the filtrate concentrated. Crystallization of the residue from hexanes/ethyl acetate (10/1) yielded a white crystalline product, N-trityl-L-valine-mercaptopyridine ester (I-1), 18.4 g, 20% yielded from valine.

Anal. Calc. for $C_{29}H_{28}NOS \cdot$ 0.1 hydrate:
C, 76.65; H, 6.26; N, 6.16.

Found: C, 76.30; H, 6.36; N, 6.14.

3-Bromomethyl-4-phenyl-1-butene (I-2)

75 mL of 0.2 molar benzyl magnesium chloride in tetrahydrofuran (Aldrich, equivalent to 0.15 moles) and 2.86 g cuprous iodide was stirred at 0°C under argon for 15 minutes then at room temperature for 25 minutes. The mixture was added to a -60°C solution of 21.4 g (0.1

mole) 1,4-dibromo-2-butene in 150 mL anhydrous diethyl ether. The mixture was stirred at -60°C for 30 minutes then at room temperature for 16 hours. The mixture was poured into 400 mL 50% saturated ammonium chloride/ice and extracted with ether. The organic layer was dried (sodium sulfate) and concentrated to a yellow oil, 21.2 g. NMR shows 30% dibenzyl present, along with the product 3-bromomethyl-4-phenyl-1-butene (I-2) estimated at 70%.

6-Tritylamino-7-methyl-3-benzyl-1-octen-5-one (I-4)

4.4 g magnesium in 150 mL dry tetrahydrofuran was treated with 0.1 mL ethylene dibromide and a catalytic amount of iodine. 10 mL of a solution of 3-bromomethyl-4-phenyl-1-butene (I-2) (28 g of 70% pure, equivalent to 19.6 g, 87 mmol) in 70 mL dry tetrahydrofuran was added. The mixture was heated, under argon to 50-60°C. After reaction started, the remaining halide was added while maintaining a 50-60°C reaction temperature. The mixture was stirred at gentle reflux for 2 hours then cooled to room temperature, to give the Grignard reagent (4-phenyl-buten-1-yl-3-methyl magnesium bromide) (I-3). 28 g (62 mmol) N-trityl-L-valine mercaptopyridine ester (I-1) was added all at once. The mixture was stirred at 50-60°C for 2 hours. The mixture was cooled to 0°C, poured into saturated ammonium chloride, and extracted with ether. The organic layer was dried (magnesium sulfate) and concentrated. Flash chromatography on silica gel (0.5% ethyl acetate in hexane) allowed

separation of diastereomers, Isomer A elutes first, Isomer B second. The total yield for both isomers is 19.1 g.

Isomer A

Mass spectroscopy yields expected MH at 486.

Anal. Calc. for $C_{35}H_{37}NO$: C, 86.20; H, 7.65; N, 2.87.

Found: C, 86.18; H, 7.67; N, 2.85.

0.82 (d, 3H), 1.03 (d, 3H), 2.08 (m, 2H), 2.10-2.65 (m, 4H) 3.16 (m, 1H) 3.26 (m, 1H) 4.70 (dd, 1H), 4.84 (dd, 1H), 5.42 (m, 1H), 6.97-7.56 (m, 20H) CH&N given for Isomer A.

Isomer B

0.84 (d, 3H), 1.10 (d, 3H), 2.00 (m, 2H), 2.20-2.65 (m, 4H) 3.05 (m, 1H), 3.30 (m, 1H) 4.76 (t, 1H) 4.90 (dd, 1H) 5.42 (m, 1H) 6.97-7.54 (m, 20H).

Isomer A 10.0 grams, TLC (0.5% EtOAc in n-hexane) R_f 0.50; isomer B 9.1 grams, R_f 0.40.

5-N-tert-Butyloxycarbonylamino-6-methyl-2-benzyl-4-oxo-heptanoic acid (I-7)

1.06 g (2.17 mmol) 6-Tritylamino-7-methyl-3-benzyl-1-octen-5-one (I-4, ß diastereomer) and 0.46 g (2.39 mmol, 1.1 eq) p-toluene-sulfonic acid in 50 mL acetonitrile was stirred at room temperature for 1 hour. Concentration yielded a white residue. Trituration with hexanes/ether (3/2, 50 mL) yielded a white solid product 6-amino-7-methyl-3-benzyl-1-octen-5-one p-toluenesulfonic

acid salt (I-5), 0.8 g (88% theory).

A solution of 0.8 g (1.9 mmol) 6-amino-7-methyl-3-benzyl-1-octen-5-one tosylate (I-5) and 0.84 g (3.8 mmoles) di-tert-butyl dicarbonate in 50 mL dichloromethane was treated, dropwise over 10 minutes, with 0.21g (2.1 mmol) triethylamine in 2 mL dichloromethane. The solution was stirred at room temperature for 16 hours. The solution was washed with 0.5 N hydrochloric acid, then water, then 1 M sodium carbonate, then water. The organic layer was dried (sodium sulfate) and concentrated. Flash chromatography on silica gel (50% hexane/dichloromethane) yielded a colorless syrup N-tert-butyloxycarbonyl-6-amino-7-methyl-3-benzyl-1-octen-5-one (I-6), 0.4 g, 61% theory.

0.27 g N-tert-butyloxycarbonyl-6-amino-7-methyl-3-benzyl-1-octen-5-one (I-6, 0.78 mmol) in 10 mL acetone was cooled to 0°C and treated with a 0°C solution of 0.9 g (4.2 mmol) sodium metaperiodate and 10.5 mg $RuO_2 \cdot xH_2O$ (59.27% Ru) in 5 mL water. The mixture was stirred at room temperature for 1 hour then filtered through Celite. The Celite pad was washed with acetone. The filtrate and washes were combined and saturated with sodium chloride. The biphasic mixture was extracted with chloroform (filtering of emulsions was necessary). The organic phase was washed with 10% sodium bisulfite, dried over sodium sulfate and concentrated to yield 5-N-tert-butyloxycarbonylamino-6-methyl-2-benzyl-4-oxo-heptanoic

acid (I-7) as a light brown foamy solid, 0.18 g (64% theory). Mass spectroscopy yields expected M+1 at 364.

Anal. Calc. for $C_{20}H_{20}NO_5$ 0.15 Hydrate: C, 65.60; H, 8.06; N, 3.82.

Found: C, 65.61; H, 7.95; N, 3.74.

Isomer A of 5-N-tert-butyloxycarbonylamino-6-methyl-2-benzyl-4-oxo-heptanoic acid attachment to Merrifield chloromethyl resin (I-8 Isomer A)

Isomer A of the ketomethylene subunit I-7 (0.500 g, 1.38 mmol) was dissolved in a mixture of MeOH and water (50 mL, 9:1). Solid cesium bicarbonate (0.268 g, 1.38 mmol) was added to the mixture and the solution stirred at room temperature for 1 hour. The solvent was removed under reduced pressure and the resulting residue was evaporated from toluene three times (50 mL each) to remove residual water, then placed under high vacuum overnight. The ketomethylene cesium salt was dissolved in DMF (50 mL) and Merrifield chloromethyl resin (2.01 g, 0.75 meq/g resin) was added to the solution. The coupling was stirred under an argon atmosphere at 50°C for 48 hours. The reaction was cooled to room temperature and filtered, and the resin washed exhaustively with methanol, methylene chloride, and isopropanol. The resin was rinsed with diethyl ether and dried under vacuum for 24 hours. This provided the desired derivatized resin I-8 (Isomer A) (2.29 g, 0.437 meq/g resin). Unreacted ketomethylene subunit

(0.110 g, 0.302 mmol) was recovered from the washes.

Isomer B of 5-N-tert-butyloxycarbonylamino-6-methyl-2-benzyl-4-oxo-heptanoic acid attachment to Merrifield chloromethyl resin (I-8 Isomer B)

Isomer B of the ketomethylene subunit I-7 (1.60 g, 4.41 mmol) was treated with cesium bicarbonate (0.857 g, 4.42 mmol) in the same manner as that described above. Coupling to Merrifield chloromethyl resin (9.02 g, 0.75 meq/g resin), yielded the desired derivatized resin I-8 (Isomer B) (10.2 g, 0.349 meq/g resin).

N^{α}-(N^{α}-Butyloxycarbonyl-N^{ε}-2-chlorobenzyloxycarbonyl-L-lysyl)-L-aspartic acid β-cyclohexyl ester (I-9)

N^{α}-Butyloxycarbonyl-N^{ε}-2-chlorobenzyloxycarbonyl-L-lysine (2.08 g, 5.0 mmol) and N-hydroxy succinimide (0.634 g, 5.5 moles) were dissolved in methylene chloride (25 mL) and cooled to 0°C (ice-water bath). DCC (1.256 g, 6.0 mmol) in methylene chloride was added dropwise over a 5-minute period. The reaction was stirred at 0°C for 3 hours, then stored at 4°C overnight. The material was filtered and the filtrate evaporated to a solid. The trifluoroacetate salt of aspartic acid β-cyclohexyl ester, prepared by reacting N^{α}-Boc aspartic acid β-cyclohexyl ester (2.21 g, 7.0 mmol) with 50% TFA in methylene chloride (40 mL) for 1 hour and evaporating the mixture to a solid, was dissolved in 50% aqueous THF and the pH was adjusted to 8 with N-methylmorpholine. The succinimydyl ester was dissolved in THF (10 mL) and added

to the aspartic acid solution. The pH was adjusted to 8 using N-methylmorpholine and the reaction was allowed to stir overnight. The THF was removed under reduced pressure and the crude material partitioned between diethyl ether (200 mL) and 5% sodium bicarbonate (200 mL). The bicarbonate layer was acidified carefully to pH 3 and extracted with three portions of ethyl acetate (100 mL each). The combined organic layers were dried over sodium sulfate, filtered, and evaporated to a solid foam. The material was purified using silica gel and filter-pad chromatography--elutions were performed utilizing a step gradient of 0-10% ethanol in ethyl acetate containing 0.25% acetic acid. Pure fractions were pooled and evaporated to a solid foam to give 2.33 g (76%) of N^{α}-(N^{α}-Butyloxycarbonyl-N^{ε}-2-chlorobenzyloxycarbonyl-L-lysyl)-L-aspartic acid ß-cyclohexyl ester (I-9);

R_f 0.23 (5% EtOH, 0.25% AcOH in EOAc);

^{1}H-NMR ($CDCl_3$) δ 1.2-1.95 (m, 19H), 2.87 (m, 2H), 3.18 (m, 2H), 4.18 (m, 1H), 4.75 (m, 3H), 5.20 (s, 2H), 5.42 (m, 1H), 7.35 (m, 4H);

Anal. Calc. for $C_{29}H_{42}N_3O_9Cl$: C, 56.90; H, 6.92; N, 6.86.

Found: C, 57.00; H, 7.20; N, 6.84.

<u>Solid phase synthesis of 5-N-(L-arginyl-L-lysl-L-asparty1)-amino-6-methyl-2-benzyl-4-oxo-heptanoic acid ((IV) F^5-THP, Isomer A)</u>

Following the general Procedure of Almquist et al.

(1988), Boc-Lys (ClZ)Asp (cHex) (I-9) (1.22 g, 2.0 mmoles) and HOBt hydrate (0.310 g, 2.03 mmol) were dissolved in DMF (5 mL) diluted with methylene chloride (5 mL) and cooled to 0°C in an ice bath. DCC (0.446 g, 2.16 mmol) in methylene chloride (10 mL) was added dropwise over 5 minutes. The reaction was stirred in methylene chloride for 15 minutes followed by treatment with 40% TFA/10% anisole/50% CH_2Cl_2 for 30 minutes to remove the Boc group. During the activation of the dipeptide, ketomethylene-derivatized Merrifield resin (I-8a) (2.29 g, 0.437 meq/g resin) was treated with 40% TFA/10% anisole in methylene chloride (5 minutes, then 30 minutes) to remove the Boc group. The peptide-resin was washed alternately with methylene chloride and isopropanol to wash out residual TFA. The activated dipeptide solution (prepared from I-9 above) was filtered, and added to the peptide-resin along with diisopropylethylamine (0.35 mL, 2.0 mmol). The reaction was shaken at room temperature. The reaction was determined to be complete (negative Kaiser test) after 5 hours. The peptide-resin was washed successively with methylene chloride (3x), isopropanol (2x), and methylene chloride (3x). The Boc group was removed by treatment of the peptide-resin with 40% TFA/10% anisole in methylene chloride (5 minutes, then 30 minutes) and the resin was washed to remove TFA. N^{α}-Boc-N^{g}-Tosyl-arginine (0.857 g, 2.0 mmol) was converted to its HOBt ester using HOBt

hydrate (0.316 g, 2.06 mmol) and DCC (0.448 g, 2.17 mmol) following the same procedure as described above for I-9. The activated ester was added to the resin along with diisopropylethylamine (0.350 mL, 2.0 mmol) and the reaction vessel was shaken overnight. The reaction was checked for completion (negative Kaiser test), and the peptide-resin was successively washed with methylene chloride (3x), isopropanol (2x), methylene chloride (2x), and methanol (2x). The peptide-resin was placed under vacuum overnight to give 3.04 g of dry peptide-resin. The Boc group was removed as described above and the resin washed successively with methylene chloride (3x), isopropanol (2x), methylene chloride (2x), and methanol (2x). The peptide-resin was dried under vacuum for 3 hours, then treated with anisole (3 mL) in anhydrous HF (30 mL) for 90 minutes at 0°C. The HF/anisole was removed under vacuum and the resin was washed with anhydrous diethyl ether. Crude N-(L-arginyl-L-lysyl-L-asparty1)-5-amino-6-methyl-2-benzyl-4-oxo-heptanoic acid ((IV) F^5-THP, Isomer A) was extracted from the resin using 10% acetonitrile in water with 0.5% TFA (four 25 mL extractions). The material was partially evaporated to remove acetonitrile, then frozen and lyophilized. The product was purified by preparative HPLC using a 5-25% gradient of acetonitrile in water containing 0.1% TFA. Pure fractions (k'=3.35 mL on a Vydac 218TP5415 column eluting at 1 mL/min with 20% acetonitrile/0.1% TFA in

water) were pooled, evaporated of acetonitrile, frozen, and lyophilized--yield 308.3 mg (31%).

FAB-MS: 663 m/e ($M+H^+$).

Anal. Calc. for $C_{31}H_{50}N_8O_8 \cdot 3CF_3CO_2H$: C, 44.23; H, 5.31; N, 11.15; F, 17.02.

Found: C, 44.04; H, 5.17; N, 11.06; F, 16.77.

Solid phase synthesis of 5-N-(L-arginyl-L-lysyl-L-aspartyl)-amino-6-methyl-2-benzyl-4-oxo-heptanoic acid ((IV) F^5-THP, Isomer B)

Using the same techniques employed to synthesize the (IV) F^5-THP, (Isomer A), I-9 (1.84 g, 3.0 mmol), and HOBt hydrate (0.460 g, 3.0 mmol) were coupled using DCC (0.680 g, 3.30 mmol) and the resulting activated ester was added along with diisopropylethylamine (0.525 mL, 3.0 mmol) to the TFA salt of deprotected ketomethylene-derivatized Merrifield resin I-8 (4.10 g, 0.349 meq/g resin). The reaction was determined to be complete (negative Kaiser test) after 4 hours. Following removal of the Boc group, the peptide-resin was washed and reacted 2.5 hours with the HOBt ester of N^{α}-Boc-N^{g}-tosyl arginine, prepared by reacting the amino acid derivative (0.857 g, 2.0 mmol) with HOBt hydrate (0.316 g, 2.06 mmol) and DCC (0.448 g, 2.17 mmol) in the presence of diisopropylethylamine (0.525 mL, 3.0 mmol). The peptide-resin was washed and stored under vacuum overnight to give 4.98 g of dry peptide-resin. Removal and recovery of the peptide from resin was performed in a manner analogous to the method

employed for Isomer A. The product was purified by preparative HPLC using a 0-25% gradient of acetonltrile in water containing 0.1% TFA. Pure fractions (k'=4.43 mL on a Vydac 218TP5415 column eluting at 1 mL/min with 20% acetonitrile/0.1% TFA in water) were pooled, evaporated of acetonitrile, frozen, and lyophilized--yield 320.0 mg (21%).

FAB-MS: 663 m/e ($M+H^+$).

Anal. Calc. for $C_{31}H_{50}N_8O_8 \cdot 3.5CF_3COOH \cdot H_2O$:

C, 42.26; H, 5.18; N, 10.38; F, 18.47.

Found: C, 42.36; H, 5.17; N, 10.60; F, 18.00.

Example 2

Preparation of Arg-Nle-Asp-Ala(k)Phe ((IV) $Nle^2A^4F^5$-THP)

N-Trityl-L-alanine (II-2)

L-Alanine (40.0 g, 0.45 mol) and chlorotrimethylsilane (48.8 g, 0.45 mol) in a mixture of chloroform (640 mL) and acetonitrile (160 mL) were stirred at reflux under argon for 2 hours. The mixture was cooled to 0°C and treated dropwise with triethylamine (90.9 g, 0.90 mol), then dropwise with a solution of triphenylmethyl chloride (125.45 g, 0.45 mol) in chloroform (400 mL). The mixture was stirred at room temperature for 1 hour, then quenched by adding methanol (450 mL). After evaporation to a foam, the solid was partitioned between ice-cold 5% citric acid (1000 mL) and diethyl ether (1500 mL), and the organic layer was extracted with

1N NaOH (2×1000 mL). The combined aqueous layers were neutralized with acetic acid and extracted with ethyl acetate (3×500 mL). The organic extracts were combined, dried over $MgSO_4$, and evaporated to give 133.8 g (90%) of II-2.

^1H NMR ($CDCl_3$) δ 1.21 (d, 3H), 3.32 (m, 1H), 6.45 (bs, 1H), 7.00-7.36 (m, 15H).

N-Trityl-L-alanine-2-mercaptopyridine ester (II-3)

DCC (9.1 g, 44.1 mmol) was added to a solution of II-2 (14.2 g, 42.8 mmol) and 2-mercaptopyridine (4.75 g, 42.8 mmol) in ethyl acetate (160 mL) and the resulting mixture stirred 4 days at room temperature. The DCU was filtered off and the filtrate evaporated to give a light yellow solid. The material was crystallized to give 4.18 g (23%) of II-3.

^1H-NMR ($CDCl_3$) δ 0.98 (d, 3H), 2.36 (d, 1H), 3.56 (m, 1H), 7.00-7.60 (m, 18H), 8.40 (m, 1H).

6-Trityl-amino-3-benzyl-1-hepten-5-one (II-6)

The Grignard reagent II-5 was prepared as described previously (I-3, Example 1) using halide II-4 (2.2 g, 9.86 mmol) and Mg turnings (500 mg) in THF (50 mL). The reaction was initiated using a crystal of iodine and refluxed 2 hours. Upon cooling to room temperature, a solution of II-3 (4.18 g, 9.86 mmol) in THF (50 mL) was added dropwise over 15 minutes. The resulting mixture was stirred at 65°C for 2 hours, then at room temperature overnight. The solution was partitioned between diethyl

ether (400 mL) and saturated NH_4Cl (400 mL). The organic layer was washed with saturated brine, dried over $MgSO_4$, and evaporated to give a pale yellow syrup. The material was purified by flash chromatography eluting with 2.5% ethyl acetate in hexane to give 1.98 g (43.8%) of II-6 as a colorless syrup.

^1H NMR ($CDCl_3$) δ 1.15 (m, 3H), 1.50 (m, 1H), 2.00 (m, 1H), 2.22 (m, 1H), 2.46 (m, 2H), 3.21 (m, 2H), 4.60-4.88 (m, 2H), 5.28-5.72 (m, 1H), 6.95-7.52 (m, 20H).

6-tert-Butyloxycarbonylamino-3-benzyl-1-hepten-5-one (II-7)

Compound II-6 (1.88 g, 3.98 mmol) was dissolved in acetonitrile (50 mL) and treated with p-toluene sulfonic acid monohydrate (832 mg, 3.98 mmol), and the resulting solution was stirred at room temperature for 2 hours. The solvent was removed, and the material was suspended in CH_2Cl_2 (50 mL). Di-tert-butyl dicarbonate (1.76 g, 7.96 mmol) and triethylamine (0.44 g, 7.96 mmol) were added to the solution and the reaction stirred at room temperature overnight. The solution was washed with 0.5 N HCl, water, 1M sodium carbonate and water. The organic layer was dried over Na_2SO_4, then evaporated to an oil. Flash chromatography on silica gel using methylene chloride as the eluant gave 0.98 g (77.6%) of II-7 as a colorless oil.

90MHz ^1H NMR ($CDCl_3$) δ 1.36 (d, 3H), 1.52 (s, 9H), 2.69 (m, 2H), 2.74 (m, 2H), 3.06 (m, 1H), 4.62

(m, 1H), 4.90-5.42 (m, 3H), 5.62-6.02 (m, 1H), 7.32 (m, 5H).

5-tert-Butyloxycarbonylamino-4-oxo-2-benzyl-hexanoic acid (II-8)

An aliquot (4 mL) of a solution containing sodium metaperiodate (4.86 g, 22.6 mmol) in water (20 mL) was used to dissolve $RuO_2 \cdot xH_2O$ (6.4 mg, 59.3% Ru). A second aliquot (4 mL) of the metaperiodate solution was added to a solution of compound II-7 (900 mg, 2.84 mmol) in acetone (30 mL), and the resulting mixture was stirred at room temperature. The ruthenium-metaperiodate solution was added dropwise to the mixture over a 5-minute period. The remaining periodate solution was then added dropwise over a 10-minute period, and the reaction was stirred at room temperature for 3 hours. The mixture was filtered through a pad of Celite and the pad washed with acetone (2×30 mL). The acetone was removed under vacuum and the resulting aqueous solution diluted with water (80 mL) and saturated with NaCl. The aqueous material was extracted with CH_2Cl_2 (3×100 mL), the extracts dried over Na_2SO_4, and the resulting material evaporated to a brown gum. Flash chromatography on silica gel using 4% MeOH in chloroform afforded 603 mg (63.3%) of II-8 as an oil.

R_f 0.32 (5% MeOH in $CHCl_3$).

^1H NMR ($CDCl_3$) δ 1.20-1.60 (m, 12H), 2.65 (m, 1H), 2.90 (m, 2H), 3.27 (m, 2H), 4.28 (m, 1H), 5.02 (bs, 1H), 7.33 (m, 5H), 9.12 (bs, 1H).

5-tert-Butyloxycarbonylamino-4-oxo-2-benzyl-hexanoic acid attachment to Merrifield chloromethyl resin (II-9)

Using an adaptation of the procedure previously described (I-8, Example 1), II-8 (570 mg, 1.70 mmol) and cesium bicarbonate (330 mg, 1.70 mmol) were mixed overnight in absolute methanol (50 mL). The mixture was evaporated and the resulting solid was dissolved in DMF (50 mL). Merrifield chloroform resin (3.40 g, 3.4 mmol @ 1 meq/g resin) was added, and the suspension was stirred under argon at 50°C for 48 hours. TLC of the DMF showed no residual ketomethylene. The resin was washed numerous times with methylene chloride and isopropanol, then dried, providing 3.90 g (99% theory) of II-9.

N^{α}-tert-Butyloxycarbonyl-L-norleucyl-L-aspartic acid ß-benzyl ester (II-10)

By a procedure analogous to the dipeptide preparation previously described (I-9, Example 1), Boc-L-norleucine succinimide (3.90 g, 11.8 mmol) was reacted with aspartic acid ß-benzyl ester (2.68 g, 12 mmol) to give 3.30 g (66%) of II-10.

Anal. Calcd. for $C_{22}H_{32}O_7N_2$: C, 60.53; H, 7.39; N, 6.35.

Found: C, 60.78; H, 7.41; N, 6.35.

Solid-phase synthesis of N-(L-arginyl-L-norleucyl-L-aspartyl)-5-amino-2-benzyl-4-oxo-hexanoic acid, Isomer A and B ((IV) $Nle^{2}A^{4}F^{5}$-THP)

Using the procedure described previously ((IV)

F^5-THP, Isomer A; Example 1), II-10 (1.10g, 2.55 mmol), DCC (578 mg, 2.80 mmol) and HOBt (390 mg, 2.55 mmol) were combined to prepare the activated ester. After removal of the Boc group from resin II-9 (3.90 g, 1.70 mmol), the activated ester and diisopropylethylamine (0.46 mL, 2.55 mmol) were added to the resin and the reaction was shaken overnight. The coupling was judged complete (Kaiser test), and the Boc group was removed. N^{α}-Boc-N^{g}-Tosyl-L-arginine (1.45 g, 3.40 mmol), DCC (773 mg, 3.75 mmol), and HOBt (520 mg, 3.4 mmol) were combined, and the resulting activated ester was added along with diisopropylethylamine (0.61 mL, 3.4 mmol) to the resin. After being shaken for 4 hours, the coupling was complete (Kaiser test). The resin was washed and dried; yield, 4.68 g. A portion of the resin (2.5 g) was treated with TFA, then with HF, to remove the peptide from the resin, yielding 380 mg of crude peptide. The product was partially purified by preparative HPLC using a 10-30% gradient of acetonitrile in water containing 0.1% TFA to give mixtures of the two isomers relatively free of other contaminants. The isomers were separated using preparative HPLC under 24% acetonitrile isocratic conditions. Pure fractions were pooled for each isomer, and each was evaporated of acetonitrile and lyophilized, which produced 46 mg (98% pure) of isomer A of (IV) $Nle^2A^4F^5$-THP (k'=1.25 with 25% acetonitrile) and 25 mg (96% pure, 4% isomer A) of isomer B of (IV) $Nle^2A^4F^5$-THP

(k'=1.52 with 25% acetonitrile).

Isomer A

FABS-MS m/e 620 ($M+H^+$).

Anal. Calcd. for $C_{29}H_{45}O_8N_7 \cdot 2CF_3COOH \cdot H_2O$:
C, 45.78; H, 5.70; N, 11.32; F, 13.16.

Found: C, 45.60; H, 5.56; N, 11.13; F, 10.60.

Isomer B

FABS-MS m/e 620 ($M+H^+$).

Anal. Calcd. for $C_{29}H_{45}O_8N_7 \cdot 2CF_3COOH \cdot 2H_2O$:
C, 44.84; H, 5.81; N, 11.09.

Found: C, 45.09; H, 5.91; N, 10.78.

Example 3

Preparation of Arg-Nle-Asp-Val(k)Phe ((IV) Nle^2F^5-THP)

Solid-phase synthesis of N-(L-arginyl-L-norleucyl-L-aspartyl)-5-amino-6-methyl-2-benzyl-4-oxo-heptanoic acid (Isomer A, (IV) Nle^2F^5-THP)

Following the procedure described previously (Isomer A, (IV) F^5-THP, Example 1), N^{α}-tert-Butyloxycarbonyl-L-norleucyl-L-aspartic acid ß-benzyl ester (III-4, 1.30 g, 3.0 mmol), DCC (660 mg, 3.20 mmol), and HOBt (460 mg, 3.0 mmol) were combined to prepare the activated ester. Following removal of the Boc group from resin III-2 (Isomer A, 3.25 g, 1.5 mmol), the activated ester and diisopropylethylamine (0.52 mL, 3.0 mmol) were added to the resin and the reaction was shaken overnight. The coupling was judged complete and the Boc group was removed. N^{α}-Boc-N^{g}-Tosyl-L-arginine (1.90 g, 3.0 mmol),

DCC (670 mg, 3.25 mmol), and HOBt (462 mg, 3.0 mmol) were combined and the resulting activated ester was added along with diisopropylethylamine (0.53 mL, 3.0 mmol) to the resin. After overnight shaking, the coupling was incomplete. N^{α}-Boc-N^{g}-Tosyl-L-arginine (1.86 g, 3.0 mmol), DCC (670 mg, 3.25 mmol), and HOBt (465 mg, 3.0 mmol) were combined and the resulting material coupled with the resin overnight. Coupling was judged complete after the second treatment. The Boc group was removed and the resin was washed and dried to yield 3.76 g. HF removal of peptide from the remaining resin (2.78 g) yielded 580 mg of crude peptide. The product was purified by preparative HPLC using a 15-35% gradient of acetonitrile in water containing 0.1% TFA. Pure fractions (k'=1.67 with 30% acetonitrile) were pooled, evaporated free of acetonitrile, frozen, and lyophilized to yield 88 mg (100% pure) of isomer A of (IV) $Nle^{2}F^{5}$-THP. Impure fractions were lyophilized and stored for further purification.

FABS-MS m/e 648 ($M+H^{+}$) 357 ($M-291+H^{+}$).

^{1}H NMR (D_2O) δ 0.74 (d, 3H), 0.83 (t, 3H), 0.86 (d, 3H), 1.27 (m, 4H), 1.61 (m, 2H), 1.70 (m, 2H), 1.89 (q, 2H), 2.24 (m, 1H), 2.76 (m, 2H), 2.85 (m, 2H), 2.94 (m, 2H), 3.07 (m, 1H), 3.19 (t, 2H), 4.01 (t, 1H), 4.29 (t, 2H), 4.35 (d, 1H), 4.70 (dd, 1H), 7.20-7.37 (m, 5H).

^{13}C NMR (D_2O) δ 16.32, 19.04, 21.77, 23.50, 27.20, 28.14, 29.37, 30.95, 35.47, 37.20, 40.50, 41.50,

42.02, 50.12, 52.60, 54.09, 63.65, 127.02, 128.81, 129.18, 138.22, 156.83, 169.32, 171.92, 173.51, 173.88, 178.92, 210.00.

Anal. Calcd. for $C_{31}H_{49}O_8N_7 \cdot 2.3CF_3COOH$: C, 46.98; H, 5.68; N, 10.78; F, 14.41.

Found: C, 47.01; H, 5.86; N, 10.78; F, 14.18.

Solid-phase synthesis of N-(L-arginyl-L-norleucyl-L-aspartyl)-5-amino-6-methyl-2-benzyl-4-oxo-heptanoic acid (Isomer B, (IV) Nle^2F^5-THP)

Following the procedure described previously (Isomer A of (IV) F^5-THP, Example 1), N^{α}-tert-Butyloxycarbonyl-L-norleucyl-L-aspartic acid ß-benzyl ester (III-4, 1.30 g, 3.0 mmol), DCC (660 mg, 3.20 mmol), and HOBt (460 mg, 3.0 mmol) were combined to prepare the activated ester. Following removal of the Boc group from resin III-2 (Isomer B, 3.20 g, 1.5 mmol), the activated ester and diisopropylethylamine (0.52 mL, 3.0 mmol) were added to the resin and the reaction was shaken overnight. The coupling was judged complete and the Boc group was removed. N^{α}-Boc-N^{g}-Tosyl-L-arginine (1.90 g, 3.0 mmol), DCC (670 mg, 3.25 mmol), and HOBt (462 mg, 3.0 mmol) were combined and the resulting activated ester was added along with diisopropylethylamine (0.53 mL, 3.0 mmol) to the resin. After shaking overnight, the coupling was incomplete. N^{α}-Boc-N^{g}-Tosyl-L-arginine (1.86 g, 3.0 mmol), DCC (670 mg, 3.25 mmol), and HOBt (465 mg, 3.0 mmol) were combined and the resulting material

coupled with the resin overnight. Coupling was judged complete after the second treatment. The Boc group was removed and the resin was washed and dried to yield 3.95 g. HF removal of peptide from the resin yielded 824 mg of crude peptide. The product was purified by preparative HPLC using a 15-29% gradient of acetonitrile in water containing 0.1 TFA. Pure fractions (k'=2.89 with 26% acetonitrile) were pooled, evaporated of acetonitrile, frozen, and lyophilized to yield 167 mg (100% pure) and 145 mg (95% pure) of isomer B of (IV) $Nle^{2}F^{5}$-THP.

FABS-MS m/e 648 ($M+H^{+}$) 357 ($M-291+H^{+}$).

^{1}H NMR (D_2O) δ 0.76 (d, 3H), 0.82 (t, 3H), 0.83 (d, 3H), 1.28 (m, 4H), 1.63 (m, 2H), 1.70 (m, 2H), 1.89 (m, 2H), 2.17 (m, 1H), 2.71 (m, 1H), 2.75 (m, 1H), 2.80 (m, 1H), 2.85 (m, 2H), 2.93 (m, 1H), 3.06 (m, 1H), 3.19 (t, 2H), 4.01 (t, 1H), 4.23 (d, 1H), 4.29 (t, 1H), 4.70 (dd, 1H), 7.20-7.36 (m, 5H).

^{13}C NMR (D_2O) δ 16.48, 18.88, 21.78, 23.50, 27.22, 28.16, 29.23, 30.96, 35.39, 37.04, 40.52, 40.85, 42.07, 50.09, 52.60, 54.11, 64.26, 127.03, 128.84, 129.17, 138.29, 156.82, 169.35, 171.98, 173.51, 173.81, 178.84, 210.59.

Anal. Calcd. for $C_{31}H_{49}O_8N_7 \cdot 2.25CF_3CO_2H$: C, 47.15; H, 5.69; N, 10.84; F, 14.18.

Found: C, 47.03; H, 5.55; N, 10.46; F, 13.96.

Example 4

Preparation of Arg-Nle-Asp-Val (CHOH)Phe

((IV) Nle^2F^5-THP($CHOHCH_2$))

N-(L-Arginyl-L-norleucyl-L-aspartyl)-5-amino-6-methyl-4-hydroxy-2-benzyl-heptanoic acid lactone ((IV) Nle^2F^5-THP-lactone, Isomer A)

Compound Isomer A of compound (IV) Nle^2F^5-THP (78 mg, 90 µmol) in methanol (1.0 mL) was treated dropwise with 0.2 M lithium borohydride in THF (5.0 mL, 1.0 mmol). The reaction was followed by analytical HPLC. After 20 minutes, the reaction was judged complete and the material evaporated twice from methanol (25 mL each). HPLC analysis indicated that the material existed in a 19:1 ratio of open hydroxy acid to lactone. This material was combined with the crude isomer A of (IV) Nle^2F^5-THP (CHOH) (10.5 mg, 12.0 µmol) prepared similarly in a test reaction, and the combined materials were purified on HPLC using a 15-26% gradient of acetonitrile in water containing 0.1% TFA. Under the acidic conditions of preparative HPLC, the equilibrium between isomer A of (IV) Nle^2F^5-THP (CHOH) and its lactone favors the lactone. A second HPLC purification using a 15-26% gradient of acetonitrile in water containing 0.1% TFA was performed to separate the two forms of isomer A of (IV) Nle^2F^5-THP (CHOH). As with the first purification, pure fractions of isomer A of (IV) Nle^2F^5-THP (CHOH) immediately began to lactonize. These fractions were

pooled, evaporated of acetonitrile, and lyophilized to give 26 mg of a mixture of isomer A of (IV) Nle^2F^5-THP (CHOH) (k'=1.37 using 26% acetonitrile) and isomer A of (IV) Nle^2F^5-THP-lactone. Fractions containing pure lactone were pooled, evaporated of acetonitrile, and lyophilized to give 54 mg (100% pure) of isomer A of (IV) Nle^2F^5-THP-lactone (k'=3.90 using 26% acetonitrile).

Isomer A of (IV)-Nle^2F^5-THP-lactone.

FAB-MS m/e 632 ($M+H^+$).

N-(L-Arginyl-L-norleucyl-L-aspartyl)-5-amino-6-methyl-4-hydroxy-2-benzyl-heptanoic acid ((IV) Nle^2F^5-THP ($CHOHCH_2$), Isomer A)

Isomer A of (IV)-Nle^2F^5-THP-lactone (8.8 mg, 10 µmol) was dissolved in water (2 mL) and 1N NaOH (40 µl, 40 µmol) added to adjust the pH to 9. The saponification of the lactone ring was followed by analytical HPLC and was determined to be complete within 30 minutes. Carbon dioxide (gas) was bubbled through the solution until the pH was reduced to 8. The material was frozen and lyophilized to give isomer A of (IV) Nle^2F^5-THP ($CHOHCH_2$).

Example 5

Preparation of Ac-Arg-Pro-Asp-Val(k)Phe ((IV) Ac-P^2F^5-THP

Solid-phase synthesis of N-(N^α-acetyl-L-arginyl-L-prolyl-L-aspartyl)-5-amino-6-methyl-2-benzyl-4-oxo-heptanoic acid ((IV) Ac-P^2F^5-THP, Isomer A)

N^{α}-tert-Butyloxycarbonyl-L-prolyl-L-aspartic acid ß-benzyl ester V-3 . The procedure was analogous to the dipeptide preparation previously described (1-9, Example 1). Boc-L-proline succinimide (1.56 g; 5 mmol) was reacted with aspartic acid ß-benzyl ester (1.33 g; 6 mmol) to give 1.20 g (57%) of V-3, Anal. Calcd. for $C_{21}H_{28}N_2O_7$: C 60.05; H, 6.61; N, 6.87. Found: C, 59.97; H, 6.66; N, 6.66 .

Following the procedure described previously (isomer A of (IV) F^5-THP, Example 1), V-3 (1.26 g, 3.0 mmol), DCC (650 mg, 3.15 mmol), and HOBt (460 mg, 3.0 mmol) were combined to prepare the activated ester. Following removal of the Boc group from resin V-2 (isomer A, 3.50 g, 1.5 mmol), the activated ester and diisopropylethyl-amine (0.52 mL, 3.0 mmol) were added to the resin and the reaction was shaken overnight. The coupling was judged complete (Kaiser test) and the Boc group was removed. N^{α}-Boc-N^{g}-Tosyl-L-arginine (1.90 g, 3.0 mmol), DCC (670 mg, 3.25 mmol), and HOBt (462 mg, 3.0 mmol) were combined and the resulting activated ester was added along with diisopropylethylamine (0.53 mL, 3.0 mmol) to the resin. After shaking overnight, the coupling was incomplete (positive Kaiser test). N^{α}-Boc-N^{g}-Tosyl-L-arginine (1.86 g, 3.0 mmol), DCC (670 mg , 3.25 mmol), and HOBt (465 mg, 3.0 mmol) were combined and the resulting material again coupled with the resin overnight. Coupling was judged complete after the second treatment. The Boc group was

removed and the resin was washed and dried to yield 3.98 g. A portion of this resin (994 mg) was neutralized with 5% diisopropylethylamine in methylene chloride and washed (3x) with methylene chloride. The resin was treated with acetic anhydride (2 mL) and pyridine (0.25 mL) in methylene chloride (8 mL) for 2 hours. The resin was washed and dried to give 960 mg. The peptide was cleaved by treating the resin with 10% anisole in anhydrous HF for 90 minutes at 0°C. HF was evaporated under vacuum and the peptide was extracted with 10% acetonitrile in water containing 0.5% trifluoroacetic acid. Lyophilization gave 231 mg of crude material. The product was purified by preparative HPLC using a 14-20% gradient of acetonitrile in water containing 0.1% TFA. Pure fractions (k'=0.77 with 28% acetonitrile) were pooled, evaporated of acetonitrile, frozen, and lyophilized to yield 62 mg (98% pure), 75 mg (90% pure) of isomer A of (IV) Ac-$P^{2}F^{5}$-THP.

FAB-MS m/e 674 ($M+H^{+}$).

Anal. Calcd. for $C_{32}H_{47}N_{7}O_{9}\cdot 1.25CF_{3}COOH$: C, 50.76; H, 5.96; N, 12.01; F, 8.73.

Found: C, 49.79; H, 5.96; N, 11.95; F, 8.48.

Example 6

Preparation of Arg-D-Lys-Asp-Val(k)Phe ((IV) $K^{\dagger 2}F^{5}$-THP)

Solid-phase synthesis of N-(L-arginyl-D-lysyl-L-asparty1)-5-amino-6-methyl-2-benzyl-4-oxo-heptanoic acid

(Isomer A, (IV) $K^{\dagger 2}F^{5}$-THP)

N^{α}-(N^{α}-Boc-N^{ε}-2-ClZ-D-lysyl)-L-aspartic acid ß-benzyl ester (VI-2). N^{α}-Boc-N^{ε}-2-ClZ-D-lysine (1.66 g; 4.0 mmol) and N-hydroxy succinimide (0.49 g; 4.2 mmol) were dissolved in methylene chloride (30 mL) and cooled to 0°C (ice bath). DCC (0.87 g; 4.2 mmol) was added, and the reaction was stirred at 0°C for 2 hours, then overnight at 20°C. The material was filtered and evaporated, then dissolved in a minimal amount of acetonitrile. The succinimide ester was added to a solution of L-aspartic acid ß-benzyl ester (0.98 g; 4.4 mmol) and N-methylmorpholine (0.48 mL; 4.4 mmol) in an acetonitrile/water-mixed solvent system (30 mL of 9:1). The reaction was allowed to proceed overnight. The acetonitrile was removed under reduced pressure, and the resulting material was diluted with water (50 mL). The aqueous material was acidified with dilute HCl and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with saturated brine (2×100 mL), dried over $MgSO_4$, and evaporated to give 1.85 g (78%) of VI-2. R_f 0.33 (6% MeOH, .3% AcOH in CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.55 (m, 6H), 3.03 (m, 2H), 3.16 (m, 2H), 4.23 (m, 1H), 4.92 (m 1H), 5.02 (s, 2H), 5.20 (s, 2H), 5.25 (bs, 1H), 7.30 (m, 5H).

Following the procedure described previously (Isomer A of (IV) F^{5}-THP, Example 1), VI-2 (620 mg, 1.0 mmol), DCC (220 mg, 1.05 mmol), and HOBt (161 mg, 1.05

mmol) were combined to prepare the activated ester. After removal of the Boc group from resin VI-1 (isomer A, 1.20 g, 0.5 mmol), the activated ester and N-methylmorpholine (0.12 mL, 1.05 mmol) were added to the resin and the reaction was shaken overnight. The coupling was judged complete (Kaiser test) and the Boc group was removed. N^{α}-Boc-N^{g}-Tosyl-L-arginine (642 mg, 1.5 mmol), DCC (330 mg, 1.6 mmol), and HOBt (230 mg, 1.5 mmol) were combined and the resulting activated ester was added along with diisopropylethylamine (0.27 mL, 1.5 mmol) to the resin. After being shaken for 4 hours, the coupling was complete (Kaiser test). The Boc group was removed and the resin was washed and dried to yield 1.35 g. HF removal of peptide from the resin yielded 149 mg of crude peptide. The product was purified by preparative HPLC using a 10-21% gradient of acetonitrile in water containing 0.1% TFA. Fractions containing the product were combined and lyophilized. A second preparative HPLC purification using a 10-21% gradient of acetonitrile gave pure product. Fractions (k'=1.35 with 21% acetonitrile) were pooled, evaporated of acetonitrile, frozen, and lyophilized to yield 59 mg (100% pure) of isomer A of (IV) $K^{\dagger 2}F^{5}$-THP. Impure fractions were lyophilized and stored for further purification.

FABS-MS, m/e 663 ($M+H^{+}$).

^{1}H NMR (D_2O) δ 0.76 (d, 3H), 0.86 (d, 3H), 1.37 (m, 2H), 1.62 (m, 4H), 1.74 (m, 2H), 2.24 (m, 1H),

2.66-2.79 (m, 2H), 2.80-2.87 (m, 2H), 2.90-3.02 (m, 4H), 3.04 (m, 1H), 3.19 (t, 1H), 4.00 (t, 1H), 4.23 (t, 1H), 4.36 (dd, 1H), 4.70 (dd, 1H), 7.18-7.37 (m, 5H).

^{13}C NMR (D_2O) δ 17.40, 19.87, 23.02, 24.73, 27.35, 28.93, 30.24, 31.26, 36.93, 38.10, 40.08, 41.30, 42.67, 43.05, 51.37, 53.83, 55.21, 64.62, 127.87, 127.94, 129.73, 129.95, 130.11, 157.76, 170.54, 173.09, 174.42, 175.24, 180.09, 211.29.

Anal. Calcd. for $C_{31}H_{50}O_8N_8 \cdot 2.85CF_3COOH \cdot 2H_2O$: C, 43.05; H, 5.61; N, 10.94; F, 15.88.

Found: C, 42.45; H, 5.19; N, 11.08; F, 15.41.

Example 7

Preparation of Arg-Ala-Asp-Val(k)Phe ((IV) A^2F^5-THP)

Solid-phase synthesis of N-(L-arginyl-L-alanyl-L-aspartyl)-5-amino-6-methyl-2-benzyl-4-oxo-heptanoic acid (Isomer A, (IV) A^2F^5-THP)

N^{α}-(N^{α}-Boc-L-alanyl)-L-aspartic acid ß-benzyl ester VII-2. N^{α}-Boc-L-alanine (5.67 g; 30 mmol) and N-hydroxysuccinimide (3.62 g; 31.5 mmol) were dissolved in THF (15 mL) and the resulting solution cooled to 0°C (ice bath). DCC (6.80 g; 33.0 mmol) in THF (10 mL) was added dropwise with vigorous stirring. The reaction was stirred at 0°C for 2 hours, then at room temperature overnight. The precipitated DCU was filtered off, and the filtrate was evaporated to give an oily gum. Trituration with ether gave 6.59 g (72.3%) of the succinimide ester. The succinimide ester (6.06 g; 20.0

mmol) was added to a solution of L-aspartic acid ß-benzyl ester (4.46 g; 20.0 mmol) and triethylamine (2.80 mL; 20.0 mmol) in 50% aqueous DMF (40 mL) and stirred at room temperature overnight. The reaction was poured over ice, and the pH was adjusted to 2 with 1 M HCl. The aqueous suspension was extracted with ethyl acetate (3×100 mL). The combined organic extracts were washed with 0.1 M HCl (1×150 mL), dried over Na_2SO_4, evaporated to an oil, and triturated with hexane/ether to give 2.13 g (27%) of VII-2. ^1H NMR ($CDCl_3$) δ 1.32 (d, 3H), 1.39 (s, 9H), 2.96 (m, 2H), 4.14 (m, 1H), 4.80 (m, 1H), 5.08 (s, 2H), 5.79 (s, 2H), 7.15 (d, 1H), 7.29 (s, 5H).

Following the procedure described previously (isomer A of (IV) F^5-THP, Example 1), VII-2 (394 mg, 1.0 mmol), DCC (227 mg, 1.1 mmol), and HOBt (153 mg, 1.0 mmol) were combined to prepare the activated ester. After removal of the Boc group from resin VII-1 (isomer A, 1.20 g, 0.5 mmol), the activated ester and diisopropylethylamine (0.18 mL, 1.0 mmol) were added to the resin and the reaction was shaken for 6 hours. The coupling was judged complete (Kaiser test) and the Boc group was removed. N^{α}-Boc-N^{g}-Tosyl-L-arginine (429 mg, 1.0 mmol), DCC (227 mg, 1.1 mmol), and HOBt (153 mg, 1.0 mmol) were combined and the resulting activated ester was added along with diisopropylethylamine (0.18 mL, 1.0 mmol) to the resin. After being shaken for 3 hours, the coupling was complete (Kaiser test). The Boc group was removed and the resin

was washed and dried to yield 1.39 g. HF removal of peptide from the resin yielded 176 mg of crude peptide. The product was partially purified by preparative HPLC using a 15-28% gradient of acetonitrile in water containing 0.1% TFA. Fractions containing the product were combined and lyophilized. A second preparative HPLC purification using a 15-28% gradient of acetonitrile gave pure product. Fractions (k'=0.69 with 25% acetonitrile) were pooled, evaporated of acetonitrile, frozen, and lyophilized to yield 59 mg (100% pure) of isomer A of (IV) A^2F^5-THP. Impure fractions were lyophilized and stored for further purification.

FABS-MS m/e 606 ($M+H^+$).

^1H NMR (D_2O) δ 0.75 (d, 3H), 0.86 (d, 3H), 1.37 (d, 3H), 1.64 (m, 2H), 1.90 (m, 2H), 2.23 (m, 1H), 2.71-2.84 (m, 4H), 2.92 (m, 1H), 2.96 (m, 1H), 3.08 (m, 1H), 3.19 (t, 2H), 4.00 (t, 1H), 4.36 (m, 2H), 4.67 (dd, 1H), 7.20-7.37 (m, 5H).

^{13}C NMR (D_2O) δ 17.25, 17.54, 19.88, 24.38, 28.98, 30.18, 36.59, 38.12, 41.42, 42.46, 43.01, 50.57, 51.22, 53.54, 64.66, 127.93, 129.72, 130.09, 139.14, 157.74, 170.14, 173.17, 175.12, 175.18, 180.04, 211.16.

Anal. Calcd. for $C_{28}H_{43}O_8N_7 \cdot 2CF_3CO_2H \cdot 2H_2O$:
C, 44.18; H, 5.67; N, 11.27; F, 13.10.

Found: C, 44.02; H, 5.36; N, 11.03; F, 11.78.

Example 8

Preparation of Arg(k)Nle-Asp-Val-Phe ((I) Nle^2F^5-THP)

N^{α}, N^{δ}, N^{ω}-Z_3-L-Arg-CH_2Cl (VIII-1) Step 1: To a solution of Z_3-L-Arg (10.0 g, 17.4 mmol) in THF (50 mL) cooled with a H_2O-ice bath was added NEt_3 (1.94 g, 19.0 mmol) followed at once with isobutyl/chloroformate (2.59 g, 19.0 mmol). In 20 min the $NEt_3 \cdot HCl$ was removed by filtration and washed with THF (15 mL). Ethereal CH_2N_2 (~25 mmol in 40 mL Et_2O) was added. Reaction, initially at 0°C, was allowed to warm to room temperature and stir 63 hr. Within the first 1.5 hr at 0°C, a copious precipitate formed which redissolved at room temperature. The reaction mixture was rotoevaporated to white solid, partitioned between $CHCl_3/H_2O$ and washed with saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$), evaporated, and the residue recrystallized twice from EtOAc-petroleum ether (PE) to obtain N^{α}, N^{δ}, N^{ω}-Z_3-L-Arg-CHN_2 (5.93 g, 57%): mp 123-124°C; $[\alpha]_D$-15.2 (C=1.0, DMF); ^1H NMR δ ($CDCl_3$) 8.06, 8.02, 7.98 (s, 15H, $C_6\underline{H}_5CH_2$-), 5.93 (s, 1H, -$\underline{CH}N_2$), 1.45-1.90 (mult., 6H, -$(CH_2)_3$-); IR(kBr) 2105 cm^{-1} (-$C\underline{H}N_2$) .

Following the literature method of Aplin et al. for acid sensitive haloketones, the diazoketone (9.85 g, 16.4 mmol) was dissolved in 80% aq. AcOH (250 mL) containing anhydrous LiCl (35 g, 0.83 mol) at 0°C. The mixture was allowed to warm to ambient temperature overnight. Addition of ~3 volumes H_2O and ~75 mL EtOAc with vigorous stirring precipitated white solid, collected by filtration and dessicator-dried, overnight. Recrystallization

from THF-Et_2O-PE gave VIII-1 (6.18 g, 62%): mp 132-134°C; $[\alpha]_D$-17.2° (C=1.0, DMF).

Anal. Calcd. for $C_{31}H_{33}ClN_4O_7$: C, 61.13; H, 5.46; N, 9.20; Cl, 5.82. Found: C, 61.19; H, 5.28; N, 8.79; Cl, 5.23.

tert-Butyl-2-tert-butyloxycarbonyl-2-butyl-4-oxo-5(S)-5-benzyloxycarbonyl-amino-8-dibenzyloxycarbonyl-guanidino octanoate (VIII-2)

To a suspension of NaH, 50% in mineral oil (135 mg, 2.8 mmol) in DME (25 mL), was added di-tert-butyl malonate (603 mg, 2.8 mmol) and the resulting solution was stirred under argon until it clarified. Z_3-Arg-CH_2Cl (1.53 mg, 2.5 mmol) and sodium iodide (380 mg, 2.54 mmol) were suspended in DME and stirred for 15-20 minutes. During this time most of the material dissolved and NaCl precipitated. The malonate solution was then transferred to the iodomethyl ketone solution via a cannula. TLC indicated that the reaction was complete after 1 hour. The reaction was transferred via cannula to a flask containing NaH, 50% in mineral oil (150 mg, 3.13 mmol). When the transfer was complete, 1-iodobutane (3.45 g, 18.8 mmol) was added to the mixture. After 3 hours, TLC indicated that the reaction was complete. The reaction was quenched with a few drops of AcOH and the DME was removed in vacuo. The crude material was dissolved in CH_2Cl_2 (100 mL), washed with 0.1 N HCl (2×50 mL) and saturated NaCl (250 mL), dried over $MgSO_4$, and

evaporated. The oily residue was flash-chromatographed (40×300 mm), eluting the hexanes (500 mL), EtOAc-hexanes (1:9, 1000 mL), and EtOAc-hexanes (1:3, 1000 mL). Interesting fractions were pooled and concentrated to give 1.442 g (68%) of VIII-2.

R_f 0.58 (EtOAc/hexane; 3.5:6.5);

^1H NMR ($CDCl_3$) δ 0.85 (t, 3H, CH_3), 1.15 (m, 4H), 1.43 (s, 18H, CO_2tBu), 1.45-1.98 (m, 6H), 3.05 (s, 2H, $COCH_2$), 3.91 (m, 2H, Arg δ CH_2), 4.26 (m, 1H, Arg α (CH), 5.02 (s, 2H, $ArCH_2$), 5.11 (s, 2H, $ArCH_2$), 5.20 (s, 2H, $ArCH_2$), 5.74 (d, 1H, NH), 7.30 (s, 5H, ArH), 7.36 (s, 10H, ArH), 9.31 (bd, 2H, NH);

^{13}C NMR ($CDCl_3$) δ 13.78, 22.83, 26.62, 27.70, 32.52, 42.22, 44.12, 56.31, 59.61, 66.92, 68.93, 81.39, 126.89, 127.71, 127.89, 128.30, 128.79, 134.59, 136.31, 136.80, 155.72, 160.43, 163.73, 169.73, 169.64, 205.94.

2-Butyl-4-oxo-5(S)-5-benzyloxycarbonyl-amino-8-dibenzyloxycarbonyl-guanidinooctanoate (VIII-3)

Compound VIII-2 (1.44 g, 1.7 mmol) was treated with TFA (20 mL) for 45 minutes then evaporated to a thick gum. The residual material was evaporated twice from heptane. Pyridlne (20 mL) was added to the diacid, the reaction flask was immersed in an oil bath preequilibrated at 100°C, and the reaction was heated for 30 minutes. The pyridine was removed under reduced pressure, the residue was dissolved in CH_2Cl_2 (100 mL) and washed with 1 N HCl (1×50 mL) and saturated NaCl (2×50 mL), dried

over $MgSO_4$, and evaporated. The material was purified and the diastereomers were separated by flash chromatography (25×250 mm) eluting with $CHCl_3$ (500 mL), $CHCl_3$-MeOH (98:2, 500 mL), $CHCl_3$-MeOH (96:4, 1000 mL), and $CHCl_3$-MeOH (94:6, 1000 mL). Fractions for the two isomers were pooled and evaporated, giving 286 mg of isomer A of VIII-3 and 314 mg of isomer B of VIII-3.

Isomer A

R_f 0.62 (MeOH-$CHCl_3$; 1:9);

^1H NMR ($CDCl_3$) δ 0.89 (t, 3H, $C\underline{H}_3$), 1.12-1.98 (m, 10H), 2.32 (m, 1H, $C\underline{H}CO_2H$), 2.92 (m, 2H, $COC\underline{H}_2CH$), 3.5-4.15 (m, 2H, Arg δ $C\underline{H}_2$), 4.43 (m, 1H, Arg α $C\underline{H}$), 5.09 (s, 2H, $ArC\underline{H}_2$), 5.13 (d, 2H, $ArC\underline{H}_2$), 5.25 (s, 2H, $ArC\underline{H}_2$), 5.74 (d, 1H, $N\underline{H}$), 7.36 (m, 15H, $Ar\underline{H}$), 9.31 (bs, 2H, $N\underline{H}$).

Isomer B

R_f 0.57 (MeOH-$CHCl_3$; 1:9);

1^H NMR ($CDCl_3$) δ 0.89 (t, 3H, $C\underline{H}_3$), 1.05-1.90 (m, 10H), 2.46 (m, 1H, $C\underline{H}CO_2H$), 2.90 (m, 2H, $COC\underline{H}_2CH$), 3.90 (m, 2H, Arg δ $C\underline{H}_2$), 4.24 (m, 1H, Arg α $C\underline{H}$), 5.08 (s, 2H, $ArC\underline{H}_2$), 5.13 (s, 2H, $ArC\underline{H}_2$), 5.23 (s, 2H, $ArC\underline{H}_2$), 5.89 (d, 1H, $N\underline{H}$), 7.35 (m, 15H, $Ar\underline{H}$), 9.32 (bs, 2H, $N\underline{H}$).

^{13}C-NMR shows weak absorbances at 208 ppm for the ketone carbons of the mixture of the two isomers.

Arg(k)Nle-Asp-Val-Phe ((I) Nle^2F^5-THP, Isomer A and B)

Compound VIII-3 (Isomer A, 286 mg, 0.41 mmol) in methylene chloride (5 mL) and HOBt (69 mg, 0.45 mmol) in DMF (2 mL) were combined and stirred at 0°C. DCC (104

mg, 0.5 mmol) was added dropwise over a 5 minute period. The solution was stirred at 0°C for 10 minutes, then at room temperature for 15 minutes. Meanwhile, the Boc group was removed from Boc-L-Asp (OBzl)-L-Val-L-Phe resin (0.80 g of 0.52 meq/g [theoretical], 0.42 mmol), prepared by standard solid-phase peptide synthesis. The reaction was stirred in methylene chloride for 15 minutes followed by treatment with 40% TFA/10% anisole/50% CH_2Cl_2 for 30 minutes to remove the Boc group. The resin was washed numerous times with methylene chloride and isopropyl alcohol, neutralized with 10% DIEA and washed with methylene chloride. The activated ester of isomer A of VIII-3 was filtered into the resin reaction vessel and the vessel was shaken overnight. The peptide was cleaved from the resin by stirring with 10% anisole in anhydrous HF at 0-5°C for 90 minutes. After evaporation of HF, the resin was washed with anhydrous diethyl ether (250 mL) and the peptide was extracted with 20% acetronitrile in water containing 0.5% TFA (8×25 mL). The extracts containing peptide were combined and lyophilized to give 286 mg of crude material. The crude material was purified using a 90-minute linear preparative gradient of 10-40% acetonitrile in water with 0.1% TFA. Fractions were pooled and lyophilized to give 37.6 mg (98% pure) of isomer A of (I) Nle^2F^5-THP (k'=2.74 with 23% aceto-nitrile) and 31.6 mg (99% pure) of isomer B of (I) Nle^2F^5-THP (with 23% acetonitrile).

Isomer A

FABS-MS m/e 648 ($M+H^+$).

90 MHz ^1H NMR (CD_3OD) δ 0.99 (m, 9H), 1.15-1.80 (m, 9H), 1.85-2.25 (m, 3H), 2.50-3.30 (m, 8H), 4.28 (m, 2H), 4.50 (m, 1H), 4.81 (m, 1H), 7.22 (m, 5H).

Anal. Calcd. for $C_{31}H_{49}O_8N_7 \cdot 1.5CF_3COOH \cdot 3/4H_2O$: C, 48.54; H, 6.36; N, 11.66; F, 10.17.

Found: C, 48.16; H, 5.87; N, 11.38; F, 10.37.

Isomer B

FABS-MS m/e 648 ($M+H^+$).

90 MHz ^1H NMR (CD_3OD) δ 0.92 (m, 9H), 1.20-1.85 (m, 9H), 1.85-2.23 (m, 3H), 2.55-3.30 (m, 8H), 7.22 (m, 5H).

Anal. Calcd. for $C_{31}H_{49}O_8N_7 \cdot 2.1CF_3COOH$: C, 47.65; H, 5.82; N, 11.05; F, 13.50.

Found: C, 48.05; H, 5.69; N, 10.61; F, 14.03.

Arg(k)Nle-Asp-Val-Phe ((I) Nle^2F^5-THP, Isomer C and D)

Compound VIII-3 (Isomer B, 314 mg, 0.46 mmol) in methylene chloride (5 mL) and HOBt (75 mg, 0.5 mmol) in DMF (2 mL) were combined and stirred at 0°C. DCC (113 mg, 0.55 mmol) was added dropwise over a 5 minute period. The solution was stirred at 0°C for 10 minutes, then at room temperature for 15 minutes. Meanwhile, the Boc group was removed from Boc-L-Agp(OBzl)-L-Val-L-Phe resin (0.90 g of 0.52 meq/g [theoretical], 0.47 mmol), prepared by standard solid-phase peptide synthesis, by treatment with 40% TFA/10% anisole in methylene chloride for 5

minutes, then 30 minutes. The resin was washed numerous times with methylene chloride and isopropyl alcohol, neutralized with 10% DIEA and washed with methylene chloride. The activated ester of isomer B of VIII-3 was filtered into the resin reaction vessel and the vessel was shaken overnight. The peptide was cleaved from the resin by stirring with 10% anisole in anhydrous HF at 0-5°C for 90 minutes. After evaporation of HF, the resin was washed with anhydrous diethyl ether (250 mL) and the peptide was extracted with 20% acetronitrile in water containing 0.5% TFA (8×25 mL). The extracts containing peptide were combined and lyophilized to give 212 mg of crude material. The crude material was purified using a 90-minute linear preparative gradient of 10-40% acetonitrile in water with 0.1% TFA. Fractions were pooled and lyophilized to give 14.2 mg (100% pure) and 35.4 mg (90% pure) of isomer C of (I) $Nle^{2}F^{5}$-THP (k'=2.74 in 24% acetonitrile) and 34.7 mg (100% pure) and 15.8 mg (90% pure) of isomer D of (I) $Nle^{2}F^{5}$-THP (k'=1.4 in 23% acetonitrile).

Isomer C

FABS-MS m/e 648 ($M+H^{+}$), 455 ($m-193+H^{+}$), 356 ($m-292+H^{+}$).

Anal. Calcd. for $C_{31}H_{49}O_{8}N_{7}\cdot 2CF_{3}CO_{2}H\cdot H_{2}O$: C, 47.01; H, 5.98; N, 10.97; F, 12.75.

Found: C, 47.01; H, 5.90; N, 10.97; F, 12.23.

Isomer D

FABS-MS m/e 648 ($M+H^+$).

Anal. Calcd. for $C_{31}H_{49}O_8N_7$ 1.9 $CF_3COOH \cdot H_2O$: C, 47.36; H, 6.05; N, 11.12 F, 12.28.

Found: C, 46.82; H, 5.84; N, 11.52; F, 12.14.

Example 9

Preparation of Arg(k)Nle-Asp-Val-Phe-NH_2((I) Nle^2F^5-THP-NH_2)

Solid-phase Synthesis of N^{α}-(2-butyl-4-oxo-5(S)-amino-8-guanidooctanoyl-L-aspartyl-L-valyl-L-phenylalanine amide ((I) Nle^2F^5-THP-NH_2, Isomer A and B)

Following the procedure described previously (Isomer A and B of (I) F^5-THP, Example 8), compound IX-1 isomer A (Isomer A of VIII-3, Example 8) (365.5 mg, 0.53 mmol), HOBt (82.0 mg, 0.54 mmol), BOP (236.5 mg, 0.54 mmol), and N-methylmorpholine (87.5 µl, 0.80 mmol) were mixed in DMF at room temperature for 30 minutes. TFA·Asp(OBzl)-L-Val-L-Phe-MBHA resin (1.30 g, 0.56 mmol @ 0.43 meq/g), prepared by standard solid-phase peptide synthesis, was neutralized with 10% diisopropylethylamine (2x), the resin washed with methylene chloride (3x), and the activated ketomethylene added. After the reaction vessel was shaken overnight, a Kaiser test indicated that coupling was not complete. The reaction was allowed to proceed 7 days, with Kaiser tests being performed periodically. Although the Kaiser test was never

negative, the resin was washed and dried, yielding 1.58 g. HF removal of peptide from resin yielded 393 mg of crude peptide. The product was partially purified by preparative HPLC using a 10-30% gradient of acetonitrile in water containing 0.1% TFA, which gave 55 mg (98% pure) of isomer A of (I) Nle^2F^5-THP-NH_2 (k'=1.33 with 24% acetonitrile). A second preparative HPLC purification for isomer B using a 17-30% gradient of acetonitrile gave pure product. Fractions (k'=1.33 with 28% acetonitrile) were pooled, evaporated of acetonitrile, frozen, and lyophilized; yield, 70 mg (100% pure) of isomer B of (I) Nle^2F^5-THP-NH_2.

Isomer A

^1H NMR (D_2O) δ 0.83 (d, 3H), 0.83 (t, 3H), 0.96 (d, 3H), 1.24 (m, 4H), 1.48 (m, 3H), 1.67 (m, 1H), 1.93 (m, 1H), 2.06 (m, 2H), 2.73 (dd, 1H), 2.80 (m, 2H), 2.90 (dd, 1H), 2.99 (dd, 1H), 3.05 (dd, 1H), 3.13 (dd, 1H), 3.20 (t, 2H), 4.03 (d, 1H), 4.31 (dd, 1H), 4.55 (dd, 1H), 4.74 (dd, 1H), 7.21-7.37 (m, 5H).

^{13}C NMR (D_2O) δ 14.13, 18.61, 19.36, 22.84, 24.34, 27.41, 29.38, 31.02, 32.53, 36.91, 37.86, 41.46, 41.62, 42.40, 50.89, 55.70, 59.12, 60.57, 128.19, 129.75, 130.10, 137.34, 157.79, 173.23, 173.86, 175.32, 176.56, 178.72, 207.57.

Anal. Calcd. for $C_{31}H_{50}O_7N_8 \cdot 1.75CF_3COOH \cdot 2H_2O$: C, 46.97; H, 6.37; N, 12.70; F, 11.31.

Found: C, 46.78; H, 5.72; N, 12.23; F, 9.98.

Isomer B

FABS-MS m/e 647 ($M+H^+$).

^1H NMR (D_2O) δ 0.76 (d, 3H), 0.79 (d, 3H), 0.82 (t, 3H), 1.23 (m, 4H), 1.49 (m, 3H), 1.66 (m, 1H), 1.95 (dd, 1H), 2.04 (m, 1H), 2.68-2.86 (m, 4H), 2.96 (dd, 1H), 3.03 (dd, 1H), 3.17 (dd, 1H), 3.23 (t, 2H), 4.03 (d, 1H), 4.29 (dd, 1H), 4.59 (d, 1H), 4.65 (t, 1H), 7.24-7.36 (m, 5H).

^{13}C NMR (D_2O) δ 13.98, 18.06, 19.13, 22.71, 24.20, 27.09, 29.31, 30.93, 32.63, 36.33, 37.69, 41.20, 41.28, 41.96, 50.84, 55.38, 58.88, 60.28, 127.94, 129.47, 129.55, 129.95, 137.29, 157.60, 173.08, 173.61, 175.16, 176.32, 178.28, 206.58.

Anal. Calcd. for $C_{28}H_{43}O_8N_7 \cdot 1.65CF_3COOH \cdot 2.5H_2O$: C, 47.30; H, 6.44; N, 12.87; F, 10.80.

Found: C, 47.23; H, 5.85; N, 12.69; F, 10.76.

Example 10

Preparation of Arg(k)Nle-Asp-D-Val-Phe ((I) $Nle^2V^{\dagger 4}F^5$-THP)

Solid-phase synthesis of N^{α}-(2-butyl-4-oxo-5(S)-amino-8-guanidooctanoyl-L-aspartyl-D-valyl-L-phenylalanine ((I) $Nle^2V^{\dagger 4}F^5$-THP)

Following the procedure described previously (Isomer A and B of (I) F^5-THP, Example 8), compound X-1 isomer A (Isomer A of VIII-3, Example 8) (731 mg, 1.06 mmol), HOBt (164.0 mg, 1.07 mmol), BOP (473 mg, 1.07 mmol), and N-methyl-morpholine (175 µl, 1.59 mmol) were mixed in DMF

(15 mL) at room temperature for 30 minutes. TFA·Asp(OBzl)-D-Val-L-Phe-O-resin (1.10 g, 0.61 mmol [theoretical] 0.55 meq/g), prepared by standard solid-phase synthesis using Merrifield Phe-resin, was neutralized with 10% diisopropylethylamine (2x), the resin washed with methylene chloride (3x), and the activated ketomethlylene added. After the reaction vessel was shaken overnight, a Kaiser test indicated that coupling was not complete. The reaction was allowed to proceed 7 days, with Kaiser tests performed periodically. Although the Kaiser test was never negative, the resin was washed and dried, yielding 1.31 g. HF removal of peptide from resin yielded 387 mg of crude peptide. The product was partially purified by preparative HPLC using a 10-30% gradient of acetonitrile in water containing 0.1% TFA. Isomer A co-elluted with the Asp-Val-Phe byproduct. A second preparative HPLC purification for isomer B using a 17-30% gradient of acetonitrile gave pure product. Fractions (k'=2.13 with 27% acetonitrile) were pooled, evaporated of acetonitrile, frozen, and lyophilized; yield, 37 mg (97% pure) of isomer B of (I) $Nle^{2}V^{\dagger 4}F^{5}$-THP.

Isomer B

FABS-MS m/e 648 ($M+H^{+}$).

^{1}H NMR (D_2O) δ 0.61 (d, 3H), 0.69 (d, 3H), 0.80 (t, 3H), 1.21 (m, 4H), 1.49 (m, 3H), 1.65 (m, 1H), 1.82-1.95 (m, 2H), 2.05 (m, 1H), 2.71 (dd, 1H), 2.77 (dd, 1H), 2.82 (m, 1H), 2.86 (dd, 1H), 2.93 (dd, 1H), 3.04 (dd,

1H), 3.21 (t, 2H), 3.25 (dd, 1H), 4.15 (m, 1H), 4.28 (dd, 1H), 4.62-4.69 (m, 2H), 7.22-7.35 (m, 5H).

^{13}C NMR (D_2O) δ 17.53, 19.41, 22.90, 24.37, 27.26, 29.47, 31.82, 32.73, 36.77, 38.03, 41.36, 41.44, 42.03, 51.39, 55.50, 59.05, 59.49, 128.06, 129.73, 130.12, 137.79, 157.76, 173.02, 173.24, 175.07, 176.49, 178.63, 206.82.

Anal. Calcd. for $C_{31}H_{49}O_8N_7 \cdot 1.5CF_3COOH \cdot 2.5H_2O$: C, 47.27; H, 6.49; N, 11.35; F, 9.90.

Found: C, 47.25; H, 5.95; N, 11.30; F, 9.54.

Example 11

Preparation of Arg-Lys-Asp(k)Val-Phe ((III) F^5-THP)

N^{α}-Trityl-L-aspartic acid ß-cyclohexyl ester (XI-3)

N^{α}-Boc-ß-cyclohexyl-L-agpartic acid ß-cyclohexyl ester (XI-1, 23.7 g, 75.1 mmol) was dissolved in CH_2Cl_2-TFA (1:1, 200 mL) and the resulting solution was stirred for 45 minutes at room temperature. The solvent was removed under reduced pressure and the resulting oil was diluted with Et_2O (100 mL) and precipitated with heptane (100 mL). The supernatant was decanted and the residual solvent was removed in vacuo to give XI-2 as a white solid. The salt was suspended in CH_2Cl_2 (300 mL); triethylamine (10.5 mL, 75 mmol) and trimethylsilyl-chloride (33.4 mL, 262 mmol) were added, and the resulting mixture was heated at reflux for 30 minutes. The reaction was cooled to room temperature, additional triethylamine (36.8 mL, 262 mmol) was added, and the

reaction was refluxed for 45 minutes. The reaction vessel was then cooled to 0°C and MeOH (4.5 mL, 112.5 mmol) in CH_2Cl_2 (80 mL) was added dropwise over a 10 minute period. The reaction was stirred for an additional 10 minutes, then warmed to room temperature. Trityl chloride (21.0 g, 75 mmol) in CH_2Cl_2 was added and the reaction was stirred for 3 hours. MeOH (50 mL) was added to the reaction and the material was stirred at room temperature for 30 minutes. The reaction was concentrated under a vacuum to a viscous yellow oil, which was dissolved in Et_2O and extracted with 1 N NaOH (4×250 mL). The combined aqueous layers were cooled to 0°C and neutralized with solid citric acid. The product was extracted from the aqueous layer with Et_2O (5×200 mL); the extracts were washed with saturated NaCl (2×200 mL), dried over $MgSO_4$, and evaporated to a light yellow foam to give 28.8 g (84%) of XI-3.

MS m/e 413 ($M-CO_2$).

^1H NMR ($CDCl_3$) δ 1.15-1.90 (m, 11H, cHex and Asp ß C<u>H</u>), 2.56 (dd, 1H, Asp ß C<u>H</u>), 3.58 (m, 1H, Asp α C<u>H</u>), 4.70 (m, 1H, OC<u>H</u>), 7.15-7.57 (m, 15H, Ar<u>H</u>).

<u>N-Tritylaspartic acid α-2-Mercaptopyridyl-ß-cyclohexyl diester</u>

Compound XI-3 (27.5 g, 60.0 mmol) and 2-mercapto-pyridine (6.90 g, 62.0 mmol) were dissolved in EtOAc. Argon was bubbled through the solution to reduce oxidation of the thiol. The mixture was cooled to 0°C and

DCC (13.7 g, 66 mmol) in EtOAc (50 mL) was added dropwise over a 10 minute period. The reaction was kept at 0°C for 2 hours, then allowed to stir at room temperature for 2 days. The reaction was cooled and then filtered of the urea byproduct. The solvent was removed under reduced pressure to give a thick yellow oil. The product was purified using flash chromatography (40×300 mm) eluting with EtOAc-hexanes (1.5:8.5, 1000 mL). The fractions of interest were pooled and evaporated to an off-white solid, yield 27.54 g (83%) of XI-4.

R_f 0.47 (EtOAc-hexanes, 1:4).

^1H NMR ($CDCl_3$) δ 1.13 (dd, 1H, Asp ß C<u>H</u>), 1.15-1.85 (m, 10H, cHex), 2.45 (dd, 1H, Asp ß C<u>H</u>), 3.42 (d, 1H, N<u>H</u>), 3.78 (m, 1H, Asp α C<u>H</u>), 4.61 (m, 1H, OC<u>H</u>), 7.10-7.83 (m, 18H, Ar<u>H</u>), 8.63 (m, 1H, Ar<u>H</u>).

<u>Cyclohexyl 3S-3-tritylamino-4-oxo-6-isopropyl-oct-7-enoate (XI-6)</u>

The Grignard reagent was prepared by activating a suspension of Mg turnings (4.83 g, 0.2 mol) in Et_2O (30 mL) with a dropwise addition of 1,2-dibromoethane (11.8 g, 63 mmol) in Et_2O (30 mL). The addition was kept at a rate to maintain a steady reflux. The reaction was kept at reflux while a solution of 4-methyl-3-bromomethyl-1-pentene (11.2 g, 63 mmol) in Et_2O (30 mL) was added dropwise over 3 hours. The reaction was refluxed an additional hour, then cooled to room temperature. The Grignard reagent (XI-5) was added

in portions via cannula to a solution of XI-4 (11.06 g, 20 mmol) in THF (50 mL) at -10°C (ice-methanol). The reaction, followed by TLC, was judged to be complete before all of the Grignard reagent had been added. The reaction was poured into a mixture of saturated NH_4Cl (200 mL) and Et_2O (200 mL). The organic layer was washed with saturated bicarbonate (3×200 mL), saturated NaCl (2×200 mL), dried over $MgSO_4$, and evaporated to a yellow oil. The reaction was purified on a flash column (40×300 mm), eluting with EtOAc-hexanes (1:9) to give 9.62 g (89%) of XI-6.

R_f 0.78 (EtOAc-hexanes, 1:4).

^1H NMR ($CDCl_3$) δ 0.77 (m, 6H, CH_3), 1.1-1.9 (m, 12H, cHex and C<u>H</u>C<u>H</u>). 1.95-2.62 (m, 4H, Asp ß C<u>H</u>$_2$ and COC<u>H</u>$_2$), 3.54 (m, 2H, Asp α C<u>H</u> and N<u>H</u>), 4.72 (m, 1H, OC<u>H</u>), 4.75-5.02 (m, 2H, C=C<u>H</u>$_2$), 5.22-5.67 (m, 1H, -C<u>H</u>=C), 7.02-7.48 (m, 15H, Ar<u>H</u>).

^{13}C NMR ($CDCl_3$) δ 18.87, 20.28, 23.58, 25.32, 30.74, 31.49, 38.31, 42.06, 42.28, 44.06, 59.07, 59.45, 71.31, 73.05, 115.63, 115.73, 126.46, 127.81, 128.90, 138.81, 139.09, 146.24, 170.56, 209.40.

<u>Cyclohexyl 3S-3-tert-butyloxycarbonylamino-4-oxo-6-isopropyl-oct-7-enoate (XI-7)</u>

A solution of p-TsOH·H_2O (4.382 g, 23 mmol) in CH_3CN (50 mL) was added to a solution of the trityl olefin XI-6 (11.8 g, 21.9 mmol) in CH_3CN (100 mL). After stirring 30 minutes, the tosylate salt was

filtered off, suspended in CH_2Cl_2 (100 mL), treated with triethylamine (4.56 g, 44.5 mmol) and $(Boc)_2O$ (9.72 g, 44.5 mmol), and then stirred at room temperature for 3 hours. The reaction was diluted with additional CH_2Cl_2 (100 mL), washed with ice-cold 0.1 N HCl (2×100 mL), ice-cold saturated $NaHCO_3$ (2×100 mL) and saturated NaCl (2×100 mL), dried over $MgSO_4$, and evaporated. The crude material was flash-chromatographed (40×300 mm), eluting with EtOAc-hexanes (1.5:8.5). Pooling product fractions gave 6.80 g (79%) of XI-7.

R_f 0.38 (EtOAc-hexanes, 1.5:8.5).

^1H NMR ($CDCl_3$) δ 0.86 (2d, 6H, CH_3), 1.1-1.9 (m, 12H, cHex and C<u>H</u>C<u>H</u>), 1.2-1.9 (m and s at 1.46, 21H, Boc, cHex and C<u>H</u>C<u>H</u>), 260 (m, 2H, COC<u>H</u>$_2$), 2.76 (m, 2H, Asp ß C<u>H</u>$_2$), 4.36 (m, 1H, Asp α C<u>H</u>), 4.73 (m, 1H, OC<u>H</u>), 4.8-5.2 (m, 2H, C=C<u>H</u>$_2$), 5.4-5.85 (m, 2H, -C<u>H</u>=C and N<u>H</u>).

^{13}C NMR ($CDCl_3$) δ 18.82, 20.23, 23.64, 25.26, 28.30, 31.44, 35.78, 35.99, 41.35, 41.62, 44.98, 56.04, 56.25, 73.53, 80.03, 116.00, 138.81, 155.33, 170.88, 207.45, 207.56.

<u>Cyclohexyl 3S-3-tert-butyloxycarbonylamino-4-oxo-6-carboxy-7-methyloctanoate (XI-8)</u>

A solution of olefin XI-7 (1.15 g, 2.9 mmol) in acetone (40 mL) was cooled to 0°C with stirring while a solution of $NaIO_4$ (3.6 g, 16.8 mmol) and $RuO_2 \cdot xH_2O$ (59.3% Ru; 18 mg) in H_2O was added dropwise. Once addition was complete, the reaction was allowed to warm

to room temperature and stir for 2 hours. The mixture was filtered through Celite and the pad was washed with acetone. The combined filtrates were saturated with NaCl, then extracted with EtOAc (2×100 mL). The organic extracts were combined, washed with 10% sodium bisulfite (2×50 mL), H_2O (1×50 mL), dried over Na_2SO_4, and evaporated to a foam, yield 830 mg (69%) of XI-8.

^1H NMR ($CDCl_3$) δ 0.96 (m, 6H, $C\underline{H}_3$), 1.12-2.23 (m, 20H, cHex, Boc and $C\underline{H}(CH_3)_2$), 2.35-3.15 (m, 5H, Asp β CH_2, $COC\underline{H}_2C\underline{H}CO_2$), 4.45 (m, 1H, Asp α $C\underline{H}$), 4.74 (m, 1H, $OC\underline{H}$), 5.70 (bd, 1H, $N\underline{H}$).

^{13}C NMR ($CDCl_3$) δ 19.23, 20.06, 23.58, 28.19, 29.65, 31.39, 35.91, 36.97, 45.85, 56.08, 73.59, 30.20, 155.33, 170.45, 170.93, 178.90, 206.90, 207.23.

$\underline{N^{\varepsilon}\text{-Carbobenzyloxy-L-lysine t-butyl ester (XI'-2)}}$

N^{ε}-Carbobenzyloxy-L-lysine (5.60 g, 20 mmol) was suspended in a mixture of 1,4-dioxane (25 mL) and isobutylene (50 mL). Concentrated H_2SO_4 (2 mL) was added carefully to the reaction, and the reaction allowed to proceed at room temperature while employing a Dewar condenser (dry ice/acetone) to keep isobutylene from evaporating. After 4 hours, the Dewar condenser was removed and the excess isobutylene allowed to evaporate. The reaction was poured into ice-cold 1 N NaOH (200 mL) and extracted with diethyl ether (3×100 mL). The combined organic layers were washed with saturated sodium bicarbonate (2×100 mL) and saturated

brine (2×100 mL), dried ($MgSO_4$), and evaporated to XI'-2 as an oil, which was used directly; yield 3.72 g (55.3%).

^{1}NMR δ 1.46 (m, 13H), 3.19 (m, 3H), 4.85 (bs, 1H), 5.09 (s, 2H), 7.43 (s, 5H) .

N^{α}, N^{δ}, N^{ω}-Tricarbobenzyloxy-L-arginyl-N^{ε}-carbobenzyloxy-L-lysine t-butyl ester (XI'-3)

N^{α}, N^{δ}, N^{ω}-Tricarbobenzyloxy-L-argine (5.76 g, 10 mmol) was dissolved in THF (40 mL) and cooled to -10°C with an ice-methanol bath. N-Methyl-morpholine (1.05 mL, 11 mmol) and isobutylchloroformate (1.55 mL, 12 mmol) were added successively. After stirring for 15 minutes at -10°C, a solution of XI'-2 (3.72 g, 11 mmol) in THF (10 mL) was added to the reaction. The reaction was stirred an additional 2 hours, and then it was poured into a 50% saturated brine solution (200 mL) and a solid gum precipitated. The aqueous material was decanted, and the gum was dissolved in methylene chloride (200 mL), washed with saturated sodium bicarbonate (100 mL) and saturated brine (2×100 mL), dried, and evaporated to give a white solid. The material was crystallized from THF-diethyl ether to give XI'-3, yield 7.67 g (85.7%).

^{1}H NMR ($CDCl_3$) δ 1.15-1.95 (s and m, 19H), 3.07 (m, 2H), 3.91 (m, 2H), 4.37 (m, 2H), 4.95 (m, 6H), 5.09 (s, 6H), 5.22 (s, 2H), 6.07 (bd, 1H), 6.78 (d, 1H), 7.15-7.45 (m, 20H), 9.35 (bs, 2H).

N^{α}, N^{δ}, N^{ω}-Tricarbobenzyloxy-L-arginyl-N^{ε}-carbobenzyloxy-L-lysine (XI'-4)

Dipeptide XI'-3 (1.22 g, 1.35 mmol) was treated with 50% TFA in methylene chloride at room temperature for 30 minutes. The solvents were removed in vacuo, leaving a light yellow gum. Trituration of the gum with THF followed by diethyl ether addition gave a white solid. The material was crystallized twice from THF-diethyl ether to give XI'-4, yield 1.02 g (90%).

^{1}H NMR ($CDCl_3$) δ 1.20-1.80 (m, 10H), 3.07 (m, 2H), 3.85 (m, 2H), 4.38 (m, 2H), 4.90-5.25 (m, 7H), 6.03 (d, 2H), 6.84 (d, 2H), 7.15-7.35 (m, 20H), 9.35 (m, 2H).

Arg-Lys-Asp(k)Val-Phe ((III) F^5-THP, Isomer A and B)

N-Boc-L-phenylalanine resin (373 mg of 0.67 meq/g resin, 0.25 mmol) was treated with 40% TFA/10% anisole in methylene chloride for 5 minutes and then 30 minutes to remove the N-terminal Boc group. During removal of the Boc from the resin, ketomethylene dipeptide XI-8 (208 mg, 0.50 mmol), BOP (222 mg, 0.50 mmol), and HOBt (76.9 mg, 0.50 mmol) were dissolved in DMF (2 mL). The mixture was treated with N-methylmorpholine (0.9 mL, 0.82 mmol), and the resulting solution was allowed to stir for 30 minutes. The Phe-resin was washed numerous times with methylene chloride and isopropylalcohol, neutralized with 5% diisopropylethylamine in methylene chloride, and washed again with methylene chloride. The activated ester solution of XI-8 from above was added to

the resin and the reaction allowed to proceed overnight. A Kaiser test suggested that the coupling was incomplete. A second coupling was performed using more ketomethylene subunit XI-8 (312 mg, 0.75 mmol) with BOP (333 mg, 0.75 mmol), HOBt (115 mg, 0.75 mmol), and N-methylmorpholine (1.40 mL, 1.50 mmol). After allowing the activated ketomethylene dipeptide to react with the resin overnight, a Kaiser test suggested that coupling was still incomplete. The resin was therefore treated with acetic anhydride (1 mL) and pyridine (0.1 mL) in methylene chloride for 30 minutes. Next, the Boc group was removed with 40% TFA/10% anisole in methylene chloride. N^{α}, N^{δ}, N^{ω}-Tricarbobenzyloxy-L-arginyl-N^{ε}-carbobenzyloxy-L-lyslne XI'-4 (840 mg, 1.0 mmol) and HOBt (153.3 mg, 1.0 mmol) were dissolved in DMF (2 mL) in a separate flask and cooled to 0°C. Dicyclohexyl-carbodiimide (228.2 mg, 1.1 mmol) in methylene chloride (5 mL) was added and the reaction stirred at 0°C for 15 minutes, then at room temperature for 30 minutes. The peptide-resin was washed alternately with methylene chloride and isopropyl alcohol to remove residual TFA. The activated dipeptide prepared in the separate flask was added to the peptide-resin, followed by addition of diisopropylethylamine (175 µl, 1.0 mmol). The reaction vessel was then shaken overnight. The coupling was checked for completion (negative Kaiser test) and the peptide-resin washed with methylene chloride. The

peptide was cleaved from the resin by stirring with 10% anisole in anhydrous HF at 0-5°C for 1 hour. After evaporation of the HF, the resin was washed with diethyl ether and chloroform, and then the peptide was eluted with 20% acetonitrile in water with 0.5% TFA present. The extract was frozen and lyophilized to give 128 mg of crude (III) F^5-THP. The isomers were separated by HPLC using a preparative gradient of 0-30% acetonitrile in water with 0.1% TFA. The separated isomers were further purified: isomer A was chromatographed under isocratic conditions using 18% acetonitrile in water with 0.1% TFA; isomer B was chromatographed under isocratic conditions using 22% acetonitrile in water with 0.1% TFA. This resulted in a recovery of 25.4 mg (>99% pure) of isomer A of (III) F^5-THP and 20.5 mg (>90% pure) of isomer B of (III) F^5-THP.

Isomer A

FAB-MS m/e 663.

^1H NMR (D_2O) δ 0.80 (d, 6H), 1.41 (m, 3H), 1.50-1.70 (m, 4H), 1.74 (m, 2H), 1.87 (m, 2H), 2.48 (m, 1H), 2.59 (dd, 1H), 2.66 (dd, 1H), 2.81 (m, 2H), 2.93 (m, 2H), 2.98 (dd, 1H), 3.14 (dd, 1H), 3.15 (m, 2H), 4.00 (t, 1H), 4.32 (dd, 1H), 4.44 (dd, 1H), 4.59 (dd, 1H), 7.22-7.35 (m, 5H).

^{13}C NMR (D_2O) δ 18.28, 18.83, 21.31, 22.63, 25.58, 27.29, 29.54, 29.67, 33.58, 35.97, 37.51, 38.31, 39.61, 46.62, 51.66, 52.83, 53.37, 55.08, 126.21, 127.83,

128.41, 135.89, 155.91, 169.91, 168.56, 172.30, 173.82, 174.53, 175.87, 207.60.

Anal. Calcd. for $C_{31}H_{50}O_8N_8 \cdot 2.5CF_3COOH \cdot 2H_2O$: C, 43.94; H, 5.80; N, 11.39; F, 14.49.

Found: C, 44.27; H, 5.46; N, 10.74; F, 13.95.

Isomer B

^1H NMR (D_2O) δ 0.55 (d, 3H), 0.61 (d, 3H), 0.8 (m, 1H), 1.39 (m, 2H), 1.57 (m, 2H), 1.61 (m, 2H), 1.87 (m, 2H), 2.48 (m, 1H), 2.59 (dd, 1H), 2.77 (m, 1H), 2.80 (m, 1H), 2.86 (m, 1H), 2.91 (m, 3H), 3.14 (t, 2H), 3.20 (dd, 1H), 3.99 (t, 1H), 4.31 (dd, 1H), 4.58 (dd, 1H), 4.63 (dd, 1H), 7.20-7.33 (m, 5H).

^{13}C NMR (D_2O) δ 17.61, 18.70, 21.22, 22.65, 25.51, 27.28, 29.42, 29.61, 34.06, 36.27, 36.72, 38.31, 39.59, 46.35, 51.70, 52.91, 53.66, 54.61, 126.22, 127.78, 127.90, 128.33, 136.11, 155.93, 168.53, 172.17, 173.91, 174.99, 175.73, 207.64.

Example 12

Preparation of Arg-Pro-Asp(k)Val-Phe-NH_2 ((III) P^2F^5-THP-NH_2)

N^α, N^δ, N^ω-Tribenzyloxycarbonyl-L-arginyl-L-proline t-butyl ester (XII'-3)

Tribenzyloxycarbonyl arginine (2.88 g, 5.0 mmol) in THF (40 mL) was cooled to -10°C (ice-methanol) and N-methylmorpholine (0.53 mL, 5.5 mmol) and isobutyl-chloroformate (0.78 mL, 6.0 mmol) were added successively. After stirring at -10°C for 20 minutes, a solution

of proline t-butyl ester (2.31 g) in THF (10 mL) was added to the reaction followed by N-methylmorpholine (0.52 mL, 5.5 mmol). After stirring at room temperature for 2 hours, the reaction was evaporated to an oil. The crude material was dissolved in methylene chloride (100 mL), washed with 5% citric acid (2×100 mL) and saturated brine (2×100 mL), dried over $MgSO_4$, and evaporated to a foam. Purification by flash chromatography using a step gradient of 20%, 30%, and 40% ethylacetate in hexane (500 mL each) gave 3.20 g (87.7%) of XII'-3.

FABS-MS m/e 730 ($M+H^+$).

NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.5-2.25 (m, 8H), 3.52 (q, 2H), 3.98 (m, 2H), 4.32 (m, 2H), 5.065 (s, 2H), 5.12 (s, 2H), 5.22 (s, 2H), 5.56 (d, 1H), 7.23-7.45 (m, 15H), 9.30 (bs, 2H).

Anal. Calcd. for $C_{39}H_{47}O_9N_5$: C, 64.18; H, 6.49; N, 9.60.

Found: C, 64.20; H, 6.50; N, 9.67.

<u>$N^{\alpha},N^{\delta},N^{\omega}$-Tribenzyloxycarbonyl-L-arginyl-L-Proline (XII'-4)</u>

Compound XII'-3 (1.06 mg, 1.45 mmol) was dissolved in trifluoracetic acid-methylene chloride (50 mL, 1:1 ratio) and the reaction was stirred for 30 minutes at room temperature. The material was evaporated to an oil. The residue was dissolved in methylene chloride (100 mL), washed with water (50 mL) and saturated brine (2×50 mL), passed through $MgSO_4$, and evaporated to give

869 mg (92.6%) of XII'-4.

FABS-MS m/e 674 ($M+H^+$).

NMR ($CDCl_3$) δ 1.50-2.05 (m, 8H), 3.52 (m, 2H), 3.87 (m, 2H), 4.50 (m, 2H), 5.04 (s, 2H), 5.13 (s, 2H), 5.16 (s, 2H), 5.90 (d, 1H), 7.20-7.45 (m, 15H), 9.20 (bs, 2H).

Anal. Calcd. for $C_{35}H_{39}O_9N_5$: C, 62.40; H, 5.83; N, 10.40

Found: C, 62.28; H, 5.90; N, 10.41.

Solid-phase synthesis of [2-isopropyl-4-oxo-5(S)-(L-arginyl-L-prolyl)-amino-6-carboxy-hexanoyl]-phenylalanine (Isomer A, (III) P^2F^5-THP-NH_2)

The general methodology follows that previously reported (Isomer A and B, (III) F^5-THP, Example 11). Compound XII-1 (XI-8, Example 11) (668 mg, 1.62 mmol) and N-hydroxysuccinimide were dissolved in CH_2Cl_2 and cooled to 0°C (ice bath). DCC (351 mg, 1.70 mmol) was added and the reaction was stirred at 0°C for 2 hours, then refrigerated overnight. The DCU was filtered and then evaporated to give the succinimide ester. The Boc group was removed from Boc-Phe-MBHA resin (2.5 g, 1.3 mmol @ 0.5 meq/g) using the standard protocol, and the resin was neutralized and washed. The succinimide was dissolved in CH_2Cl_2 (10 mL) and added to the resin along with a catalytic amount of HOBt (5 mg). The reaction was allowed to proceed 7 days with periodic monitoring using the Kaiser test. The resin was then capped using

acetic anhydride (1 mL) and pyridine (0.2 mL) in CH_2Cl_2 (10 mL). The Boc was removed as usual, leaving the resin as the TFA salt. Meanwhile, Z_3-L-Arg-L-Pro (XII'-4, 1.05 g, 1.56 mmol) and HOBt (250 mg, 1.64 mmol) were dissolved in CH_2Cl_2 and cooled to 0°C, after which DCC (350 mg, 1.70 mmol) was added. The resin was neutralized (2x) with 5% DIEA and washed with CH_2Cl_2 (3x) just before the activated dipeptide was added. The vessel was then shaken overnight. The reaction was determined to be complete (negative Kaiser test), and the resin was washed and dried to give 2.81 g. HF removal of peptide from resin yielded 352 mg of crude peptide. The product was partially purified by preparative HPLC using a 10-25% gradient of acetonitrile in water containing 0.1% TFA. The still impure fractions were grouped into five pools that contained product. Further preparative HPLC purification was performed in an isocratic system using 22% acetonitrile in water containing 0.1% TFA. Fractions containing isomer A (k'=2.00 with 22% acetonitrile) were pooled, evaporated of acetonitrile, frozen, and lyophilized; yield, 42 mg (98% pure) of isomer A of (III) P^2F^5-THP-NH_2. Isomer B appeared unstable, giving rise to multiple peaks in the analytical HPLC.

Isomer A

FABS-MS m/e 631 ($M+H^+$)

^1H NMR (D_2O) δ 0.43, 0.58 (d, 3H), 1.45 (m,

1H), 1.68 (m, 2H), 1.91 (m, 3H), 2.00 (m, 2H), 2.33 (m, 2H), 2.72 (m, 1H), 2.79-2.91 (m, 4H), 3.19 (t, 2H), 3.00 (dd, 1H), 3.57 (m, 1H), 3.72 (m, 1H), 4.36 (t, 3H), 4.48 (dd, 1H), 4.59 (dd, 1H), 4.63 (t, 1H), 7.22-7.34 (m, 5H).

^{13}C NMR (D_2O) δ 20.03, 20.21, 24.08, 25.75, 27.95, 30.43, 30.90, 35.49, 37.71, 39.79, 41.45, 48.56, 48.99, 52.37, 55.72, 56.15, 61.48, 128.09, 129.82, 130.03, 137.96, 157.81, 169.05, 174.45, 175.59, 177.58, 178.34, 210.94.

Anal. Calcd. for $C_{30}H_{45}O_7N_8 \cdot 1.8CF_3COOH \cdot 2.5H_2O$: C, 45.51; H, 5.90; N, 12.64; F, 11.58.

Found: C, 45.62; H, 5.47; N, 12.55; F, 11.30.

Example 13

Preparation of Arg-Lys-Asp(k)Val-Tyr ((III) THP)

Solid-phase synthesis of [2-Isopropyl-4-oxo-5(S)-(L-arginyl-L-lysyl)-amino-6-carboxy-hexanoyl]-tyrosine (Isomer A and B, (III) THP)

Using an adaption of a previously mentioned method ((III) F^5-THP, Isomer A and B, Example 11), compound XIII-1 (566 mg, 1.38 mmol), HOBt (217 mg, 1.41 mmol), BOP (617 mg, 1.40 mmol), and N-methylmorpholine (467 µl, 4.27 mmol) were combined in CH_2Cl_2 (10 mL) and stirred at room temperature for 30 minutes. Meanwhile, the Boc group was removed from Boc-L-Tyr(Z)-O-Resin (2.59 g, 1.50 mmol @ 0.58 meq/g) in the usual manner, and the resin was washed with CH_2Cl_2 (3x), neutralized with 10%

DIEA in CH_2Cl_2 (2x), and washed with CH_2Cl_2 (3x). The activated ketomethylene was added to the resin and shaken for 7 days. Because there were excess equivalents of resin compared with ketomethylene, the Kaiser test was invalid. The untreated amines were capped using acetic anhydride (1.0 mL) and pyridine (0.1 mL) in CH_2Cl_2 (9 mL). The Boc group was removed as usual, leaving the resin as the TFA salt. Meanwhile, Z_3-L-Arg-N^{ε}-Z-L-Lys (XI'-4, Example 11, 1.26 g, 1.50 mmol) and HOBt (230 mg, 1.50 mmol) were dissolved in CH_2Cl_2 and cooled to 0°C, after which DCC (340 mg, 1.65 mmol) was added. The resin was neutralized (2x) with 5% DIEA and washed with CH_2Cl_2 (3x) just before the activated dipeptide was added. The vessel was then shaken overnight. The reaction was determined to be complete (negative Kaiser test), and the resin was washed and dried to give 3.43 g of peptide-resin. HF removal of peptide from resin yielded 588 mg of crude peptide. The products were separated and partially purified by preparative HPLC using a 0-17% gradient of acetonitrile in water containing 0.1% TFA. Isomer A was further purified by preparative HPLC using a 3-12% gradient of acetonitrile in water containing 0.1% TFA. Fractions containing isomer A (k'=0.88 with 13% acetonitrile) were pooled, evaporated of acetonitrile, frozen, and lyophilized; yield, 92 mg (99% pure) of isomer A of (III) THP. Isomer B was further purified by

preparative HPLC using a 7-17% gradient of acetonitrile in water containing 0.1% TFA. Fractions containing isomer B (k'=1.12 with 16% acetonitrile) were pooled, evaporated of acetonitrile, frozen, and lyophilized; yield, 31 mg (98% pure) of isomer B of (III)-THP.

Isomer A

FABS-MS m/e 679 ($M+H^+$).

^1H NMR (D_2O) δ 0.818 (d, 3H), 0.82 (d, 3H), 1.42 (m, 2H), 1.52-1.82 (m, 7H), 1.89 (q, 2H), 2.49 (m, 1H), 2.60 (dd, 1H), 2.67 (dd, 1H), 2.78-2.97 (m, 5H), 3.07 (dd, 1H) 3.17 (m, 2H), 4.03 (t, 1H), 4.34 (dd, 1H), 4.47 (dd, 1H), 4.54 (dd, 1H), 6.80 (m, 2H), 7.12 (m, 2H).

^{13}C NMR (D_2O) δ 20.12, 20.63, 23.10, 24.44, 27.38, 29.10, 31.36, 31.47, 35.39, 36.98, 39.36, 40.12, 41.12, 41.41, 48.52, 53.48, 54.64, 55.35, 56.93, 116.33, 129.49, 131.54, 155.31, 157.71, 170.36, 174.09, 175.69, 176.48, 177.61, 209.38.

Anal. Calcd. for $C_{31}H_{50}O_9N_8 \cdot 2.6CF_3COOH \cdot 3H_2O$: C, 42.24; H, 5.74; N, 10.89; F, 14.41.

Found: C, 41.90; H, 5.31; N, 10.83; F, 14.21.

Isomer B

FABS-MS m/e 679 ($M+H^+$).

^1H NMR (D_2O) δ 0.56 (d, 3H), 0.64 (d, 3H), 0.79-0.92 (m, 1H), 1.41 (m, 2H), 1.53-1.71 (m, 5H), 1.78 (m, 2H), 1.90 (q, 2H), 2.52 (m, 1H), 2.62 (dd, 1H), 2.79 (dd, 1H), 2.81-2.87 (m, 2H), 2.89 (dd, 1H), 2.96 (t,

2H), 3.18 (m, 3H), 4.02 (t, 1H), 4.34 (t, 1H), 4.57 (dd, 1H), 4.66 (dd, 1H), 6.80 (m, 2H), 7.14 (m, 2H).

^{13}C NMR (D_2O) δ 21.96, 23.18, 25.73, 27.17, 30.03, 31.81, 33.99, 34.13, 38.66, 40.07, 41.04, 42.84, 44.11, 50.82, 56.23, 57.43, 58.32, 59.20, 119.14, 132.64, 134.20, 157.95, 160.45, 173.05, 176.68, 178.54, 179.73, 180.18, 212.11.

Anal. Calcd. for $C_{31}H_{50}O_9N_8 \cdot 2.4CF_3COOH \cdot 3H_2O$: C, 42.72; H, 5.85; N, 11.13; F, 13.60.

Found: C, 42.40; H, 5.24; N, 10.68; F, 13.15.

Example 14

Solution-phase synthesis of Arg-Lys-Asp-Val(k)Phe ((IV) F^5-THP)

First, the protected dipeptide Z-Arg(NO_2)-Lys(Z) (XIV-3) was prepared in good yield by standard methods. This dipeptide was then condensed using N-hydroxysuccinimide and DCC with the benzhydryl ester of aspartic acid (XIV-4) (prepared as shown) to give XIV-5 in 84% yield. Benzhydryl ester XIV-5 was then cleaved using trifluoroacetic acid with 10% anisole to give 83% yield of XIV-6. Compound XIV-6 was then converted to its succinate active ester and condensed with TsOH·Val(k)-CH(Bzl)$CH=CH_2$ to yield 87% of XIV-7. The olefin of XIV-7 was then oxidized with RuO_2 and $NaIO_4$ to yield 34% of XIV-8. The final step in the synthesis of (IV) F^5-THP requires the removal of all the protecting groups by

hydrogenolysis in an acidic environment to yield 20% of (IV) F^5-THP.

Furthermore, in manners similar to those set forth above for Examples 1-14, the following compounds were prepared:

Arg-Pro-Asp-Val(k)Phe (Isomer A and B, (IV) P^2F^5-THP)

Isomer A

FABS-MS m/e 632 ($M+H^+$) 341 ($m-291+H^+$).

^1H NMR (D_2O) δ 0.76 (d, 3H), 0.88 (d, 3H), 1.71 (m, 2H), 1.89 (m, 1H), 1.94 (m, 2H), 2.02 (m, 2H), 2.26 (m, 1H), 2.32 (m, 1H), 2.76 (m, 1H), 2.82 (m, 2H), 2.88 (m, 1H), 2.94 (m, 2H), 3.10 (m, 1H), 3.22 (t, 2H), 3.60 (m, 1H), 3.73 (m, 1H), 4.37 (m, 2H), 4.50 (dd, 1H), 4.67 (dd, 1H), 7.22-7.38 (m, 5H).

^{13}C NMR (D_2O) δ 16.33, 19.01, 23.15, 24.75, 27.01, 29.35, 29.53, 35.47, 37.19, 40.54, 41.57, 42.03, 48.06, 50.33, 51.46, 60.50, 63.63, 127.02, 128.81, 129.08, 138.25, 156.87, 168.11, 172.17, 173.37, 173.98, 178.93, 210.04.

Anal. Calcd. for $C_{30}H_{45}O_8N_7 \cdot 2CF_3CO_2H \cdot H_2O$: C, 46.52; H, 5.85; N, 11.15; F, 13.00.

Found: C, 46.28; H, 5.14; N, 10.91; F, 12.14.

Isomer B

FABS-MS m/e 632 ($M+H^+$) 341 ($m-291+H^+$).

^1H NMR (D_2O) δ 0.74 (d, 3H), 0.81 (d, 3H), 1.68 (m, 2H), 1.86 (m, 1H), 1.92 (m, 2H), 1.97 (m, 2H), 2.15 (m, 1H), 2.29 (m, 1H), 2.69 (m, 1H), 2.78 (m, 2H), 2.84

(m, 1H), 2.90 (m, 1H), 3.04 (m, 1H), 3.18 (t, 2H), 3.57 (m, 1H), 3.70 (m, 1H), 4.23 (d, 1H), 4.34 (t, 1H), 4.46 (dd, 1H), 4.63 (dd, 1H), 7.18-7.35 (m, 5H).

^{13}C NMR (D_2O) δ 16.77, 18.83, 23.15, 24.74, 26.99, 29.21, 29.52, 35.38, 37.03, 40.52, 40.94, 42.11, 48.05, 50.33, 51.45, 60.49, 64.19, 127.02, 128.83, 129.16, 138.29, 156.83, 168.11, 172.24, 173.38, 173.86, 178.89, 210.68.

Anal. Calcd. for $C_{30}H_{45}O_8N_7 \cdot 2CF_3CO_2H \cdot H_2O$: C, 46.00; H, 5.67; N, 11.04; F, 12.84.

Found: C, 45.96; H, 5.27; N, 10.84; F, 12.38.

Arg-Leu-Asp-Val(k)Phe (Isomer A, (IV) L^2F^5-THP)

Isomer A

FABS-MS m/e 648 ($M+H^+$).

^{13}H NMR (D_2O) δ 0.74 (d, 3H), 0.87 (d, 6H), 0.90 (d, 3H), 1.59 (m, 5H), 1.90 (m, 2H), 2.23 (m, 1H), 2.69-2.85 (m, 4H), 2.85-3.22 (m, 2H), 3.08 (m, 1H), 3.19 (t, 2H), 4.01 (t, 1H), 4.36 (m, 1H), 4.39 (m, 1H), 4.70 (m, 1H), 7.21-7.37 (m, 5H) .

^{13}C NMR (D_2O) δ 17.20, 19.93, 21.98, 22.83, 24.39, 25.30, 29.07, 30.27, 36.51, 38.12, 40.88, 41.41, 42.44, 43.00, 51.11, 53.40, 53.49, 64.59, 127.93, 129.72, 130.10, 139.14, 157.74, 170.24, 172.92, 174.75, 174.98, 179.98, 211.00 .

Anal. Calcd. for $C_{31}H_{49}O_8N_7 \cdot 1.8CF_3COOH \cdot 2H_2O$: C, 46.75; H, 6.22; N, 11.03; F, 11.55 .

Found: C, 46.45; H, 5.76; N, 10.89; F, 11.48.

Ac-Arg-Nle-Asp-Val(k)Phe (Isomer A, (IV) Ac-Nle^{2}F^{5}-THP)

Isomer A

FABS-MS m/e 690 ($M+H^+$).

Anal. Calcd. for $C_{33}H_{51}O_9N_7 \cdot CF_3CO_2H \cdot H2O$: C, 51.15; H, 6.32; N, 11.93; F, 6.93.

Found: C, 51.20; H, 6.50; N, 11.92; F, 7.14.

Arg-Nle-Asp(k)Val-Phe (Isomer A and B, (III) Nle^{2}F^{5}-THP)

Isomer A

FABS-MS m/e 648 ($M+H^+$), 357 ($M-291+H^+$).

^{1}H NM (D_2O) δ 0.83 (d, 6H), 0.87 (m, 3H), 1.32 (m, 4H), 1.63 (m, 2H), 1.76 (m, 3H), 1.90 (m, 2H), 2.51 (m, 1H), 2.63 (m, 1H), 2.66 (dd, 1H), 2.83 (dd, 1H), 2.86 (dd, 1H), 3.02 (dd, 1H), 3.17 (m, 1H), 3.19 (m, 2H), 4.03 (t, 1H), 4.32 (t, 1H), 4.48 (dd, 1H), 4.65 (dd, 1H), 7.26-7.39 (m, 5H).

^{13}C NMR (D_2O) δ 12.25, 18.24, 18.80, 20.84, 22.60, 26.30, 27.24, 29.49, 29.92, 33.29, 35.82, 37.52, 39.60, 46.56, 51.65, 52.98, 53.05, 53.14, 54.81, 126.21, 127.83, 128.37, 135.75, 155.92, 168.41, 172.68, 173.48, 174.14, 175.86, 207.60.

Anal. Calcd. for $C_{31}H_{49}O_8N_7 \cdot 1.8CF_3COOH \cdot 1/2H_2O$: C, 48.21; H, 6.07; N, 11.38; F, 11.91.

Found: C, 48.01; H, 5.76; N, 11.22; F, 11.78.

Isomer B

FABS-MS m/e 648 ($M+H^+$), 357 ($M-291+H^+$).

^{1}H NMR (D_2O) δ 0.70 (d, 3H), 0.75 (d, 3H), 0.95 (m, 3H), 1.39 (m, 4H), 1.69 (m, 3H), 1.81 (m, 2H), 1.96

(m, 2H), 2.55 (m, 1H), 2.76 (dd, 1H), 2.78-3.40 (overlapping m, 4H), 3.20 (t, 2H), 3.26 (dd, 1H), 4.01 (t, 1H), 4.30 (dd, 1H), 4.61 (m, 1H), 7.12-7.23 (m, 5H).

^{13}C NMR (D_2O) δ 12.29, 17.64, 18.72, 20.85, 22.65, 26.34, 27.28, 29.40, 29.88, 33.92, 36.21, 36.69, 39.60, 46.36, 51.70, 53.27, 53.37, 54.50, 126.21, 127.89, 128.34, 136.04, 155.92, 168.47, 172.66, 173.80, 174.67, 175.61, 207.79.

Anal. Calcd. for $C_{31}H_{49}O_8N_7 \cdot 1.6CF_3COOH \cdot 1/2H_2O$: C, 48.90; H, 6.21; N, 11.69; F, 10.87.

Found: C, 48.63; H, 5.89; N, 11.92; F, 10.85.

Arg-Pro-Asp(k)Val-Phe (Isomer A and B, (III) P^2F^5-THP)

Isomer A

FABS-MS m/e 632 ($M+H^+$), 341 ($M-291+H^+$).

Isomer B

FABS-MS m/e 648 ($M+H^+$), 357 ($M-291+H^+$).

Anal. Calcd. for $C_{30}H_{45}O_8N_7 \cdot 2CF_3CO_2H$: C, 47.50; H, 5.51; N, 11.40; F, 13.26.

Found: C, 47.82; H, 5.33; N, 11.52; F, 13.33.

Example 15

Preparation of Arg-NMeNle-Asp-Val(k)Phe ((IV) N-MeNle $^2F^5$-THP)

N^α, N^δ, N^ω-Tribenzyloxycarbonyl-L-arginyl-N^α-methyl-L-norleucine tert-butyl ester (XV-3). Compound XV-1 (2.45 g; 10.0 mmol) was added to a mixture of concentrated sulfuric acid (1.5 mL) in dioxane (40 mL) and stirred for 15 min under an argon purge.

Isobutylene (30 mL) was condensed into the reaction mixture using a Dewar reflux condensor. The reaction was stirred for 5 hr, poured into ice-cold 1N NaOH (200 mL), and extracted using diethyl ether (3×100 mL). The combined ether extracts were washed with saturated $NaHCO_3$ (100 mL) and saturated NaCl (2×100 mL), dried over $MgSO_4$, and evaporated to give 1.05 g of XV-2.

N^{α}, N^{δ}, N^{ω}-Tribenzyloxycarbonyl-L-arginine (2.88 g; 5.0 mmol) in methylene chloride (50 mL) was cooled to -10°C, and triethylamine (0.68 mL; 5.0 mmol) and isobutylchloroformate (0.78 mL; 6.0 mmol) were added successively. After stirring for 20 min, a solution of XV-2 in methylene chloride (20 mL) was added to the reaction, followed by triethylamine (0.68 mL; 5.0 mmol). The reaction was allowed to come to room temperature and stirred for 4 hr. The material was evaporated to an oil and subjected to flash chromatography using a step gradient of 10, 20, and 30% ethyl acetate in hexane (500 mL each), yielding 2.56 g (33.7%) of XV-3.

DCI-MS m/e 760 ($M+H^+$).

90 MHz ^1H NMR ($CDCl_3$) δ 0.90 (m, 3H), 1.01-1.95 (m, 10H), 1.39 (s, 9H), 2.73 (s, 0.6H), 2.83 (s, 2.4H), 4.00 (m, 2H), 4.62 (m, 1H), 5.08 (s, 2H), 5.12 (s, 2H), 5.20 (s, 2H), 5.58 (d, 1H), 7.23-7.45 (m, 15H), 9.33 (s, 2H).

Anal. Calcd. for $C_{41}H_{53}N_5O_9$: C, 64.80; H, 7.03; N, 9.22.

Found: C, 64.71; H, 6.86; N, 9.10.

N^{α}, N^{δ}, N^{ω}-Tribenzyloxycarbonyl-L-arginyl-N^{α}-methyl-L-norleucine (XV-4).

Compound XV-3 (1.14 g; 1.5 mmol) was dissolved in a mixture of trifluoroacetic acid (10 mL) and methylene chloride (10 mL) and stirred for 40 min. The material was concentrated to an oil, diluted in methylene chloride (50 mL), washed with water (4×50 mL) and saturated NaCl (50 mL), dried over $MgSO_4$, and evaporated to give 940 mg (89%) of XV-4.

DCI-MS m/e 552 (M-Z-H_2O).

90 MHz ^1H NMR ($CDCl_3$) δ 0.88 (m, 3H), 1.01-1.95 (m, 10H), 2.76 (s, 0.3H), 2.86 (s, 2.7H), 3.83 (m, 2H), 4.72 (m, 1H), 5.0-5.3 (m, 6H), 5.96 (d, 1H), 7.23-7.45 (m, 15H), 8.2-9.4 (bs, 3H).

Anal. Calcd. for $C_{37}H_{45}N_5O_9$ C, 63.14; H, 6.45; N, 9.95.

Found: C, 63.41; H, 6.30; N, 9.74.

N^{α}, N^{δ}, N^{ω}-Tribenzyloxycarbonyl-L-arginyl-N^{α}-methyl-L-norleucyl-L-aspartic acid-β-cyclohexyl-α-tert-butyl ester (XV-6).

Compound XV-4 (910 mg; 1.30 mmol) in methylene chloride (10 mL) and HOBt (213 mg; 1.40 mmol) in DMF (1 mL) were combined and cooled to -10°C. DCC (310 mg; 1.50 mmol) in methylene chloride (5 mL) was added dropwise over 5 min. The reaction was stirred for 20 min at -10°C, then at room temperature for 20 min.

Aspartic acid-ß-cyclohexyl-α-tert-butyl ester (XV-5) (433 mg; 1.60 mmol) in methylene chloride (10 mL) was added to the reaction, along with N-methylmorpholine (0.18 mL; 1.60 mmol), and stirred overnight. The reaction was evaporated to an oil and subjected to flash chromatography using a step gradient of 10, 20, and 30% ethyl acetate in hexane (500 mL each). Product fractions were pooled and evaporated to give 1.04 g (85%) of XV-6.

DCI-MS m/e 957 ($M+H^+$).

90 MHz ^1H NMR ($CDCl_3$) δ 0.88 (m, 3H), 1.0-2.0 (m, 20H), 1.47 (s, 9H), 2.65-2.90 (m, 5H), 3.97 (m, 2H), 4.4-4.8 (m, 3H), 5.08 (s, 2H), 5.12 (s, 2H), 5.20 (s, 2H), 5.60 (d, 1H), 6.73 (d, 1H), 7.20-7.48 (m, 15H), 9.30 (bs, 2H).

Anal. Calcd. for $C_{51}H_{68}N_6O_{12}$: C, 64.00; H, 7.16; N, 8.78.

Found: C, 64.41; H, 7.07; N, 8.87.

<u>N^{α}, N^{δ}, N^{ω}-Tribenzyloxycarbonyl-L-arginyl-N^{α}-methyl-L-norleucyl-L-aspartic acid-8-cyclohexyl ester (XV-7)</u>

Compound XV-6 (960 mg; 1.00 mmol) was dissolved in a mixture of methylene chloride (10 mL) and trifluoroacetic acid (10 mL) and stirred 60 min at room temperature. The material was concentrated to an oil, diluted in methylene chloride (50 mL), washed to neutrality with water (4×50 mL), dried over $MgSO_4$, and evaporated to give 840 mg (93%) of XV-7.

90 MHz ^1H NMR ($CDCl_3$) δ 0.87 (m, 3H), 1.0-2.0

(m, 20H), 2.70-2.90 (m, 5H), 3.96 (m, 2H), 4.50-4.85 (m, 3H), 5.08 (s, 2H), 5.14 (s, 2H), 5.20 (s, 2H), 5.70 (d, 1H), 5.95 (d, 1H), 7.23-7.45 (m, 15H), 9.35 (bs, 2H).

Solid phase synthesis of 6-Methyl-5(S)-(L-arginyl-N^{α}-methyl-L-norleucyl-L-aspartyl)-amino-4-oxo-2-isopropyl-heptanoic acid (Isomer A)

Using the procedure described previously for the synthesis of Arg-Lys-Asp-Val(k)Phe ((IV) F^{5}-THP) in Example 1, the Boc group was removed from the Boc-Val(k)Phe-Resin (0.50 g; 0.25 meq) by treatment with a cocktail containing 40% TFA and 10% anisole in methylene chloride for 5 min, followed by a second treatment with fresh cocktail for 30 min. After removal of the Boc group, the resin was washed four times alternately with methylene chloride and isopropanol, then finally with methylene chloride (3x). Meanwhile, tripeptide XV-7 (675 mg; 0.75 mmol) and HOBt (122 mg; 0.80 mmol) were dissolved in DMF (1 mL), diluted with methylene chloride (10 mL), and cooled to 0°C (ice bath). DCC (186 mg; 0.90 mmol) was added and the resulting mixture stirred at 0°C for 25 min, then at room temperature for 30 min. The solution was added to the resin along with diisopropylethylamine (140 µl; 0.80 mmol), and the resulting mixture was shaken overnight. The coupling was judged complete by using the Kaiser test. The resin was washed with methylene chloride and isopropanol, then dried, and yielded 0.66 g.

The peptide was cleaved from the resin with concomitant removal of side-chain protecting groups by treating the peptide-resin with 10% anisole in HF (10 mL) at 0°C for 90 min, followed by vacuum distillation of the HF anisole mixture. The resin was washed with ether and the peptide extracted with 15% acetonitrile in water containing 0.5% TFA. Lyophilization of the extracts gave 92 mg of crude peptide Arg-NMeNle-Asp-Val(k)Phe ((IV) N-MeNle^{2}F^{5}-THP). The product was purified by HPLC using a preparative gradient of 10-30% acetonitrile in water with 0.1% TFA present. Fractions containing pure compound were pooled, evaporated of acetonitrile, and lyophilized to give 21 mg of Isomer A or Arg-NMeNle-Asp-Val(k)Phe ((IV) N-MeNle^{2}F^{5}-THP) (98% pure, k'=1.58, with 27% acetonitrile in water containing 0.1% TFA).

FABS-MS m/e 662 ($M+H^{+}$).

Anal. Calcd. for $C_{32}H_{51}N_{7}O_{8}\cdot 2CF_{3}CO_{2}H$: C, 48.60; H, 6.00; N, 11.02.

Found: C, 48.70; H, 5.89; N, 10.63.

400 MHz ^{1}H-NMR ($D_{2}O$) δ 0.76 (m, 3H), 0.86 (m, 6H), 1.22 (m, 2H), 1.31 (m, 2H), 1.64 (m, 2H), 1.80 (m, 1H), 1.88 (m, 3H), 2.26 (m, 1H), 2.69-2.95 (m, 7H), 3.02 (2s, 3H), 3.12 (m, 1.2H), 3.22 (m, 0.8H), 4.33 (m, 1H), 4.52 (m, 1H), 4.58 (m, 4H), 4.87 (m, 0.6H), 4.92 (m, 0.4H), 7.20-7.38 (m, 5H).

Example 16

Preparation of Arg-NMeNle-Asp-Val(k)Val

((IV) N-MeNle^{2}V^{5}THP)

7-Methyl-6-tritylamino-3-isopropyl-1-octen-5-one (XVI-4). Grignard reagent XVI-2 was prepared by the entrainment method. To magnesium turnings (1.10 g; 45.6 mmol) in ether (20 mL) was added 1,2-dibromoethane (2.70 g; 14.4 mmol) at a rate that maintained reflux. The resulting mixture was refluxed for an additional hour, followed by the dropwise addition of 3-bromomethyl-4-methyl-1-pentene (XVI-1) (2.50 g; 14.1 mmol) in ether (10 mL) over a 30-min period. Reflux was continued for 1 hour; then the mixture was cooled to room temperature. XVI-2 was transferred via cannula to a cooled (0°C) solution of XVI-3 (3.18 g; 7.5 mmol) in THF (30 mL). The reaction was allowed to proceed at room temperature overnight. The material was partitioned between diethyl ether (300 mL) and saturated NH_4Cl (300 mL). The organic layer was washed with saturated NaCl (2×200 mL), dried over $MgSO_4$, filtered, and evaporated. The material was purified with filter-pad chromatography (30 mL bed volume of silica gel) using a step gradient with carbon tetrachloride and methylene chloride as the eluants (0%, 25%, 50%, 75%, 100% methylene chloride). Product fractions were combined and evaporated to give 1.26 g (38.3%) of XVI-4 as a clear syrup.

DCI-MS m/e 440 ($M+H^+$).

90 MHz ^{1}H NMR ($CDCl_3$) δ 0.65-1.05 (m, 9H), 1.14 (dd, 3H), 1.23-1.68 (m, 2H), 1.80-2.45 (m, 3H), 3.18 (dd, 1H), 3.43 (dd, 1H), 4.77-5.12 (m, 2H), 5.20-5.72 (m, 1H), 7.18-7.65 (m, 15H).

22.5 MHz ^{13}C NMR ($CDCl_3$) δ 17.46, 18.28, 18.82, 19.14, 19.25, 19.98, 20.28, 20.77, 30.14, 31.22, 31.44, 32.36, 43.20, 43.79, 44.28, 65.52, 70.72, 70.82, 115.19, 115.95, 126.30, 127.65, 128.90, 129.17, 138.65, 139.25, 146.67, 210.43, 211.08.

Anal. Calcd. for $C_{31}H_{37}NO \cdot 0.2\ CHCl_3$: C, 80.90; H, 8.09; N, 3.02.

Found: C, 80.84; H, 7.86; N, 3.03.

<u>7-Methyl-6-tert-butyloxycarbonylamino-3-isopropyl-1-octen-5-one (XVI-5)</u>.

Compound XVI-4 (1.20 g; 2.73 mmol) was dissolved in acetonitrile (15 mL) and treated with p-toluene sulfonic acid monohydrate (860 mg; 4.5 mmol); the resulting solution was stirred for 2 hours. As the tosyl salt did not precipitate, the mixture was concentrated under vacuum to an oil. This was dissolved in methylene chloride (20 mL) and treated with di-tert-butyl dicarbonate (1.30 g; 6.0 mmol) followed by triethylamine (0.84 mL; 6.0 mmol). The reaction was stirred overnight, then evaporated to a yellow oil. Filter-pad chromatography (30 mL bed volume of silica gel) using a step gradient of carbon tetrachloride and methylene chloride (0%, 25%, 50%, 75%, 100% methylene chloride) was used to obtain 589 g (72%) of

XVI-5 as a clear syrup.

DCI-MS m/e 298 ($M+H^+$).

90 MHz ^1H NMR ($CDCl_3$) δ 0.63-1.00 (m, 12H), 1.23-1.69 (m, 2H), 1.40 (s, 9H), 2.08 (m, 1H), 2.47 (m, 2H), 4.16 (m, 1H), 4.78-5.15 (m, 3H), 5.32-5.78 (m, 1H).

22.5 MHz ^{13}C NMR ($CDCl_3$) δ 16.49, 16.55, 18.55, 18.87, 19.90, 20.01, 20.28, 28.24, 29.81, 30.03, 31.22, 31.33, 43.14, 43.58, 44.88, 45.26, 63.67, 64.43, 79.49, 116.22, 138.60, 138.81, 155.82, 208.48, 208.86.

Anal. Calcd. for $C_{17}H_{31}NO_3$: C, 68.65; H, 10.51; N, 4.71.

Found: C, 68.38; H, 10.58; N, 4.69.

6-Methyl-5-tert-butyloxycarbonylamino-4-oxo-2-isopropyl heptanoic acid (XVI-6).

An aliquot (3 mL) of a solution containing sodium metaperiodate (2.80 g; 13.0 mmol) in water (10 mL) was used to dissolve $RuO_2 \cdot xH_2O$ (3 mg; 59.3% Ru). A second aliquot (3 mL) of the metaperiodate solution was added to a solution of compound XVI-5 (486 mg; 1.63 mmol) in acetone (20 mL), and the resulting mixture was stirred at room temperature. The ruthenium-metaperiodate solution was added dropwise to the reaction over a 5-min period. The remaining periodate solution was added dropwise over a 10-min period and the reaction was stirred overnight. Isopropyl alcohol (3 mL) was added to the reaction and the mixture was stirred for 1 hour. The reaction was filtered through Celite and the Celite

was washed with acetone. The acetone was partially removed under vacuum, and the residue was suspended in saturated NaCl (100 mL) and extracted with methylene chloride (3×75 mL). The combined extracts were washed with saturated NaCl (100 mL), dried over $MgSO_4$, and evaporated. Filter-pad chromatography using 3% methanol in methylene chloride gave 458 g (89%) of XVI-6 as a light purple solid.

DCI-MS m/e 316 ($M+H^+$).

90 MHz ^1H NMR ($CDCl_3$) δ 0.60-0.98 (m, 12H), 1.34 (s, 9H), 2.02 (m, 2H), 2.45 (m, 1H), 2.72 (m, 2H), 4.20 (m, 1H), 5.16 (d, 1H), 8.72 (bs, 1H).

22.5 MHz ^{13}C NMR ($CDCl_3$) δ 16.22, 16.70, 19.30, 19.63, 19.79, 20.06, 28.14, 29.60, 29.92, 38.16, 38.97, 45.47, 45.91, 63.51, 64.11, 79.65, 156.04, 179.06, 179.28, 207.99, 208.48.

Anal. Calcd. for $C_{16}H_{29}NO_5$: C, 60.93; H, 9.27; N, 4.44.

Found: C, 60.39; H, 9.33; N, 4.46.

<u>6-Methyl-5(S)-tert-butyloxycarbonylamino-4-oxo-2-isopropyl-heptanoic acid attachment to Merrifield chloromethyl resin (XVI-7)</u>.

Using an adaptation of the procedure previously described for the synthesis of Arg-Lys-Asp-Val(k)Phe ((IV) F^5-THP) in Example 1, the ketomethylene subunit Boc-Val(k)Val-OH (XVI-6, 425 mg; 1.35 mmol) and cesium bicarbonate (281 mg; 1.45 mmol) were mixed overnight in

absolute methanol (25 mL). The mixture was evaporated and the resulting solid was dissolved in DMF (25 mL). Merrifield chloromethyl resin (2.62 g; 2.62 mmol @ 1 meq/g resin) was added and the suspension stirred under argon at 50°C for 48 hours. TLC of the DMF showed no residual ketomethylene XVI-6. The resin was washed numerous times with methylene chloride, methanol, and isopropanol, then dried, to give 2.89 g (ca 0.47 meq/g) of XVI-7.

Solid-phase synthesis of 6-Methyl-5(S)-(L-arginyl-N^{α}-methyl-L-norleucyl-L-aspartyl)-amino-4-oxo-2-isopropyl-heptanoic acid (Isomer A and Isomer B)

Using the procedure previously described for the synthesis for Arg-Lys-Asp-Val(k)Phe ((IV) F^{5}-THP) in Example 1, the Boc group was removed from the resin XVI-7 (1.0 g; 0.33 meq) by treatment with a cocktail containing 40% TFA and 10% anisole in methylene chloride for 5 min, followed by a second treatment with fresh cocktail for 30 min. After removal of the Boc group, the resin was washed three times alternately with methylene chloride and isopropanol, then finally with methylene chloride (3 times). Meanwhile, tripeptide XVI-8 (XV-7 in Example 15) (333 mg; 0.37 mmol) and HOBt (57 mg; 0.37 mmol) were dissolved in DMF (2 mL), diluted with methylene chloride (4 mL), and cooled to 0°C (ice bath). DCC (84 mg; 0.41 mmol) in methylene chloride (2 mL) was added, and the resulting mixture stirred at 0°C

for 10 min, then at room temperature for 20 min. The solution was added to the resin along with diisopropylethylamine (65 µl; 0.37 mmol), and the resulting reaction was shaken overnight. Tripeptide XVI-8 (110 mg; 0.12 mmol), HOBt (19 mg; 0.12 mmol) and DCC (28 mg; 0.14 mmol) were combined to form additional activated XVI-9. The solution was added to the resin and the reaction was shaken for 2 days. The peptide-resin was washed with methylene chloride (2 times), isopropanol (2 times) and finally methylene chloride (2 times), to give 1.317 g. The peptide was cleaved from the resin with concomitant removal of side-chain protecting groups by treating the peptide-resin with 10% anisole in HF (12 mL) at 0°C for 1 hour, followed by vacuum removal of the HF/anisole mixture. The resin was washed with ether and the peptide extracted with 50% acetic acid (50 mL). Lyophilization of the extracts gave 255 mg of crude peptide Arg-NMeNle-Asp-Val(k)Val ((IV) N-MeNle^{2}V^{5}-THP). The product was purified by HPLC using a preparative gradient of 16-27% acetonitrile in water with 0.1% TFA present. Fractions containing pure compounds were pooled, evaporated of acetonitrile, and lyophilized to give 54.6 mg of Isomer A of Arg-NMeNle-Asp-Val(k)Val ((IV) N-MeNle^{2}F^{5}-THP) (98% pure, k'=0.63, with 25% acetonitrile in water containing 0.1% TFA) and 41.1 mg of Isomer B of Arg-NMeNle-Asp-Val(k)Val ((IV) N-MeNle^{2}F^{5}-THP) (95% pure, k'=1.52, with 25%

acetonitrile in water containing 0.1% TFA).

Isomer A

FABS-MS m/e 614 ($M+H^+$).

400 MHz ^{1}H-NMR (D_2O) δ 0.78 (t, 3H), 0.85 (m, 3H), 0.91 (m, 9H), 1.20 (m, 2H), 1.31 (m, 2H), 1.66 (m, 2H), 1.70-1.97 (m, 5H), 2.60 (m, 1H), 2.80 (m, 2H), 2.86 (m, 1H), 3.00 (m, 1H), 3.02 (s, 2H), 3.05 (s, 1H), 3.23 (m, 2H), 4.42 (d, 0.3H), 4.45 (d, 0.7H), 4.56 (t, 0.3H), 4.60 (t, 0.7H), 4.74 (dd, 1H), 4.93 (dd, 1H).

100 MHz ^{13}C NMR (D_2O) δ 14.18, 17.39, 20.05, 20.49, 22.56, 22.75, 24.32, 24.56, 28.02, 28.09, 28.28, 28.64, 30.39, 30.74, 32.16, 36.56, 41.00, 41.04, 47.59, 47.66, 51.39, 51.99, 58.68, 64.74, 157.86, 171.11, 171.76, 173.42, 173.50, 175.03, 175.07, 180.66.

Isomer B

FABS-MS m/e 614 ($M+H^+$).

400 MHz ^{1}H-NMR (D_2O) δ 0.80 (d, 3H), 0.88 (t, 3H), 0.93 (m, 9H), 1.25 (m, 2H), 1.32 (m, 2H), 1.67 (m, 2H), 1.80 (m, 1H), 1.92 (m, 4H), 2.34 (m, 1H), 2.62 (m, 1H), 2.76 (dd, 1H), 2.79 (dd, 1H), 2.85 (m, 1H), 3.03 (m, 1H), 3.06 (s, 3H), 3.23 (t, 2H), 4.49 (d, 1H), 4.56 (t, 1H), 4.73 (dd, 1H), 4.91 (dd, 1H).

100 MHz ^{13}C NMR (D_2O) δ 14.31, 17.42, 20.17, 20.24, 20.61, 22.86, 24.53, 28.25, 28.45, 28.74, 30.51, 30.85, 32.67, 37.14, 41.62, 47.66, 47.72, 51.72, 51.85, 59.41, 64.74, 171.17, 173.33, 173.53, 175.77, 180.62.

Example 17

Preparation of Arg-Lys-Asp(k)Ala-Phe ((III) A^4F^5-THP)

Cyclohexyl 3-tritylamino-4-oxo-6-methyl-7-octenoate (XVII-4)

Grignard reagent XVII-2 was prepared by the entrainment method. To magnesium turnings (1.30 g; 54.0 mmol) in ether (20 mL) was added 1,2-dibromoethane (3.4 g; 18.0 mmol) at a rate that maintained reflux. The resulting mixture was refluxed for an additional hour, followed by the dropwise addition of 1-bromo-2-methyl-3-butene (XVII-1; 2.70 g; 18.0 mmol) in ether (10 mL) over a 30-min period. Reflux was continued for 1 hour; then the mixture was cooled to room temperature. The Grignard reagent was transferred via cannula to a cooled (0°C) solution of XVII-3 (3.30 g; 6.0 mmol) in THF (30 mL). After stirring at 0°C for 2 hours, the reaction was poured into a biphasic mixture of saturated NH_4Cl and diethyl ether (400 mL each). The organic layer was washed with saturated NaCl (2×200 mL), dried over $MgSO_4$, filtered, and evaporated to give crude product. The material was purified on flash chromatography using 15% ethyl acetate in hexane as the eluant to give 2.68 g (88%) of XVII-4 as a light yellow syrup.

DCI-MS m/e 510 ($M+H^+$).

90 MHz ^1H NMR ($CDCl_3$) δ 0.92 (m,3H), 1.20-2.10 (m, 11H), 2.15-2.72 (m, 4H), 3.60 (d, 1H), 3.65 (m, 1H), 5.70-6.05 (m, 3H), 4.63 (m, 1H), 7.18-7.65 (m, 15H),

22.5 MHz ^{13}C NMR ($CDCl_3$) δ 14.05, 19.14, 19.30, 22.56, 23.58, 25.26, 31.49, 31.93, 39.03, 43.41, 46.23, 59.01, 59.12, 71.26, 73.05, 112.43, 112.65, 126.46, 126.84, 127.27, 127.82, 128.84, 129.60, 142.93, 144.83, 146.13, 170.51, 209.46.

Anal. Calcd. for $C_{34}H_{39}NO_3 \cdot 0.15CHCl_3$: C, 77.74; H, 7.48; N, 2.65.

Found: C, 77.74; H, 7.50; N, 2.70.

Cyclohexyl 3-tert-butyloxycarbonylamino-4-oxo-6-methyl-7-octenoate (XVII-5)

Compound XVII-4 (2.24 g; 4.4 mmol) was dissolved in diethyl ether (30 mL) and treated with p-toluene sulfonic acid monohydrate (860 mg; 4.5 mmol); the resulting solution was stirred for 3 hours. The precipitated salt was filtered and the white solid was suspended in methylene chloride (40 mL). Di-tert-butyl dicarbonate (1.95 g; 8.8 mmol) was added to the reaction followed by triethylamine (1.22 mL; 8.8 mmol). The reaction was stirred for 4 hours, then evaporated to a yellow oil. Filter-pad chromatography using a step-gradient of carbon tetrachloride and methylene chloride (0%, 25%, 50%, 75%, 100% methylene chloride) was used to obtain 1.36 g (84%) of XVII-5 as a clear syrup.

DCI-MS m/e 368 ($M+H^+$).

90 MHz ^1H NMR ($CDCl_3$) δ 0.91 (d, 3H), 1.02-1.83 (m, 11H), 1.34 (s, 9H), 2.51 (m, 2H), 2.71 (m, 2H), 4.30 (m, 1H), 4.63 (m, 1H), 4.70-4.98 (m, 2H),

5.48-5.84 (m, 2H).

22.5 MHz ^{13}C NMR ($CDCl_3$) δ 19.52, 23.53, 25.16, 28.19, 31.33, 32.58, 35.83, 45.31, 45.47, 56.04, 73.37, 79.93, 112.97, 142.61, 155.23, 170.61, 207.07.

Anal. Calcd. for $C_{20}H_{33}NO_5$: C, 65.37; H, 9.05; N, 3.81.

Found: C, 65.45; H, 9.25; N, 3.89.

1-O-Cyclohexyl-3-tert-butyloxycarbonylamino-4-oxo-6-methyl-heptanedioic acid (XVII-6)

An aliquot (5 mL) of a solution containing sodium metaperiodate (5.85 g; 27.2 mmol) in water (20 mL) was used to dissolve $RuO_2 \cdot xH_2O$ (6 mg; 59.3% Ru). A second aliquot (5 mL) of the metaperiodate solution was added to a solution of compound XVII-5 (1.24 g; 3.4 mmol) in acetone (40 mL), and the resulting mixture was stirred at room temperature. The ruthenium-metaperiodate solution was added dropwise over the 10-min period. The remaining periodate solution was added dropwise over the 10-min period and the reaction was stirred for 4 hours. Isopropyl alcohol (5 mL) was added to the reaction and stirring was continued for an additional 3 hours. The reaction was filtered through Celite and the Celite was washed with acetone. The acetone was partially removed under vacuum, the residue was suspended in saturated NaCl (100 mL) and extracted with methylene chloride (3×100 mL). The combined extracts were washed with saturated NaCl (100 mL), dried over $MgSO_4$, and

evaporated to give 1.30 g (100%) of XVII-6 as a solid.

DCI-MS m/e 386 ($M+H^+$).

90 MHz ^1H NMR ($CDCl_3$) δ 1.18 (d, 3H), 1.20-1.93 (m, 10H), 1.40 (s, 9H), 2.53-3.18 (m, 5H), 4.42 (m, 1H), 4.70 (m, 1H), 5.68 (d, 1H), 10.28 (bs, 1H).

22.5 MHz ^{13}C NMR ($CDCl_3$) δ 16.70, 23.48, 25.10, 28.08, 31.28, 34.31, 35.78, 41.84, 55.82, 73.48, 80.09, 155.39, 170.45, 170.78, 180.53, 206.21, 206.48.

Anal. Calcd. for $C_{19}H_{31}NO_7$: C, 59.20; H, 8.11; N, 3.63.

Found: C, 59.39; H, 8.16; N, 3.75.

Solid-phase synthesis of [2-Isopropyl-4-oxo-5(S)-(L-arginyl-L-lysyl)-amino-6-carboxy-hexanoyl]-L-phenylalanine (Isomer A and Isomer B)

N-Boc-L-phenylalanine-resin (1.62 g; 1.08 mmol @ 0.67 meq/g) was treated with a cocktail comprised of 40% TFA and 10% anisole in methylene chloride for 5 min followed by a 30 min treatment with a second portion of cocktail. During the removal of the Boc group from the resin, ketomethylene XVII-6 (387 mg, 1.0 mmol), HOBt (158 mg; 1.0 mmol) in deuterated DMF (1 mL), and DCC (227 mg; 1.1 mmol) were combined in methylene chloride (10 mL), stirred at 0°C for 20 min, and then at room temperature for 20 min. The resin was washed alternately with methylene chloride and isopropanol (3x), neutralized with 10% DIEA in methylene chloride (2x) and washed with methylene chloride (3x). The activated ester of XVII-6

was added to the resin and the reaction vessel shaken four days. The resin was treated with acetic anhydride (2 mL) and pyridine (0.5 mL) for one hour. Coupling was judged complete (Kaiser test). The Boc group was removed form the resin as described above. Meanwhile, the dipeptide N^{α}, N^{δ}, N^{ω}-Z_3-L-Arg-N^{ε}-Z-L-Lys-OH (XI'-4 in Example 11) (1.13 g; 1.4 mmol) and HOBt (210 mg; 1.4 mmol) were dissolved in DMF (10 mL) and stirred at 0°C while DCC (310 mg; 1.49 mmol) was added to the reaction. The reaction was stirred for 15 min at 0°C, then for 30 min at room temperature. The resin was neutralized with 5% DIEA in methylene chloride washes (2x) followed by methylene chloride washes (3x) just prior to the addition of the activated dipeptide XVII-8. After overnight coupling, the reaction was judged complete (Kaiser test). The resin was washed with DMF (2x), methylene chloride (2x), and isopropyl alcohol (2x), and dried under vacuum to give 2.41g of peptide-resin (XVII-9). The peptide was cleaved from the resin with concomitant removal of side-chain protecting groups by treating the peptide-resin (1.21 g; 0.5 mmol) with 10% anisole in HF (12 mL) at 0°C for one hour followed by vacuum distillation of the HF/ anisole mixture. The resin was washed with ether, and the peptide extracted with 10% acetonitrile in water containing 0.5% TFA. Lyophilization of the extracts gave 396 mg of crude peptide Arg-Lys-Asp(k)Ala-Phe ((III) A^4F^5-THP) (78% theor.). The isomers were separated by

HPLC using a preparative gradient of 0-20% acetonitrile in water with 0.1% TFA present. The interesting fractions were pooled, evaporated of acetonitrile, and lyophilized to give 64 mg of isomer A or Arg-Lys-Asp(k)Ala-Phe ((III A^4F^5-THP) (99% pure, k'=0.77 with 17% acetonitrile) and 79 mg of isomer B of Arg-Lys-Asp(k)Ala-Phe ((III) A^4F^5-THP) (95% pure, k'=1.58 with 17% acetonitrile).

Isomer A

FABS-MS m/e 635 ($M+H^+$).

^{1}H-NMR (D_2O) δ 1.06 (d, 3H), 1.44 (m, 2H), 1.58-1.76 (m, 4H), 1.82 (m, 2H), 1.93 (m, 2H), 2.59 (dd, 1H), 2.70 (dd, 1H), 2.76 (dd, 1H), 2.81 (m, 2H), 2.91 (dd, 1H), 2.99 (t, 2H), 3.04 (dd, 1H), 3.21 (t, 2H), 3.23 (dd, 1H), 4.07 (t, 1H), 4.37 (t, 1H), 4.54 (dd, 1H), 4.64 (dd, 1H), 7.28-7.42 (m, 5H).

^{13}C-NMR (D_2O) δ 17.76, 23.07, 24.41, 27.34, 29.08, 31.37, 35.42, 36.29, 37.69, 40.10, 41.38, 43.05, 53.44, 54.62, 55.25, 56.66, 128.02, 129.63, 130.23, 137.73, 157.69, 170.34, 174.09, 175.48, 176.30, 179.01, 208.85.

Anal. Calcd. for $C_{29}H_{46}N_8O_8 \cdot 3CF_3CO_2H \cdot 0.5H_2O$: C, 42.64; H, 5.11; N, 11.36; F, 17.34.

Found: C, 42.55; H, 4.74; N, 11.37; F, 17.33.

Isomer B

FABS-MS m/e 635 ($M+H^+$).

^{1}H-NMR (D_2O) δ 0.90 (d, 3H), 1.43 (m, 2H), 1.63

(m, 2H), 1.69 (m, 2H), 1.79 (m, 2H), 1.92 (m, 2H), 2.61 (dd, 1H), 2.73-2.87 (m, 3H), 2.91-3.20 (m, 4H), 3.20 (t, 2H), 3.25 (dd, 1H), 4.05 (t, 1H), 4.36 (t, 1H), 4.64 (m, 2H), 7.25-7.40 (m, 5H).

^{13}C NMR (D_2O) δ 18.19, 23.00, 24.43, 27.30, 29.08, 31.35, 35.63, 36.45, 38.07, 40.12, 41.38, 43.04, 53.48, 54.69, 55.11, 56.44, 128.01, 129.62, 130.27, 137.78, 157.71, 170.33, 174.03, 175.46, 176.35, 178.89, 208.95.

Anal. Calcd. for $C_{29}H_{46}N_8O_8 \cdot 3CF_3CO_2H \cdot 0.33$N-Ac-Phe: C, 44.39; H, 5.14; N, 11.16; F, 16.36.

Found: C, 44.36; H, 5.08; N, 10.82; F, 16.36.

Example 18

Preparation of Arg-NMeLys-Asp(k)Val-Phe ((III) N-MeK^2F^5-THP)

N-[1-Methoxycarbonyl-5-(2-chlorobenzyloxycarbonyl)-aminopentyl]-2-azanorbornene (XVIII-3' and XVIII-4')

Following the procedure of Grieco and Bahsas (J. Org. Chem. 1987, 52, 5746), to N^{ε}-(2-chlorobenzyloxycarbonyl)-L-lysine methyl ester hydrochloride (XVIII-1') (14.59 g; 40.0 mmol) in water (25 mL) was added 37% formaldehyde (3.6 mL; 45.0 mmol). The solution was stirred while cyclopentadiene (XVIII-2') (15 mL; 183.0 mmol) was distilled into the reaction. The resulting biphasic mixture was stirred vigorously for 2 hours. The aqueous material was diluted with water (50 mL) and extracted with hexane (2×50 mL), then neutralized with solid sodium

bicarbonate and extracted with methylene chloride (2×100 mL). The organic extracts were washed with saturated NaCl (2×100 mL), dried over $MgSO_4$, filtered, and evaporated to give crude product. Purification on silica using 2% methanol in methylene chloride as the eluant gave 14.7 g (90%) of XVIII-3' and XVIII-4' as a yellow oil. The material was used directly.

Methyl N^{α}-tert-butyloxycarbonyl-N^{α}-methyl-N^{ε}-(2-chlorobenzyloxycarbonyl)-L-lysine (XVIII-6')

Compounds XVIII-3' and XVIII-4' (8.00 g; 19.7 mmol) were dissolved in a mixture of trifluoroacetic acid and chloroform (200 mL of 1:1 mixture). Triethylsilane (10.0 mL; 62.0 mmol) was added, and the reaction was stirred at room temperature for 18 hours under argon. The material was concentrated to an oil residue, then taken up in 0.1N HCl (100 mL) and washed with hexane (2×50 mL). The aqueous layer was neutralized with solid sodium bicarbonate and extracted with methylene chloride (3×100 mL). The organic portions were washed with saturated $NaHCO_3$ (100 mL) and saturated NaCl (2×100 mL), dried over $MgSO_4$, and evaporated to give 4.3 g of crude amine XVIII-5'. The amine was dissolved in methylene chloride (50 mL) and treated with di-tert-butyl dicarbonate (4.80 g; 22.0 mmol). After being stirred for 4 hours, the reaction was evaporated to an oil and subjected to filter-pad chromatography using a step gradient with carbon tetrachloride and methylene chloride as the

eluants (0, 25, 50, 75, 100% methylene chloride). Product factions were combined and evaporated to give 4.69 g (54%) of XVIII-6' as a clear syrup.

DCI-MS m/e 443 ($M+H^+$).

90 MHz ^{1}H-NMR ($CDCl_3$) δ 1.10-2.15 (m, 6H), 1.40 (s, 9H), 2.79 (s, 3H), 3.22 (m, 2H), 3.68 (s, 3H), 4.32 (m, 0.5H), 4.76 (m, 0.5H), 5.00 (m, 1H), 5.20 (s, 2H), 7.15-7.45 (m, 4H).

Anal. Calcd. for $C_{21}H_{31}N_2O_6Cl$: C, 56.94; H, 7.05; N, 6.32; Cl, 8.00.

Found: C, 56.66; H, 7.04; N, 6.21; Cl, 7.54.

N^{α}-tert-butyloxycarbonyl-N^{α}-methyl-N^{ε}-(2-chlorobenzyloxy-carbonyl)-L-lysine (XVIII-7')

Compound XVIII-6' (4.42 g; 10.0 mmol) was dissolved in methanol (100 mL) and cooled to 0°C (ice bath). To this mixture was added a solution of 1N NaOH (15 mL; 15 mmol). The reaction was stirred at 0°C for 2 hours, then overnight at room temperature. The reaction was concentrated to an oil and treated with a mixture of ice-cold 0.1N HCl (100 mL) and methylene chloride (100 mL). The aqueous layer was extracted with additional methylene chloride (2×100 mL), and the combined organic extracts were washed with saturated NaCl (100 mL), dried over $MgSO_4$, and evaporated to give 4.26 g (92.2%) of XVIII-7' as a clear oil.

Trimethylsilylated DCI-MS m/e 451 ($M+H^+$).

90 MHz ^{1}H-NMR ($CDCl_3$) δ 1.10-1.95 (m, 6H), 1.40

(s, 9H), 2.79 (s, 3H), 3.24 (m, 2H), 4.35 (m, 0.5H), 4.76 (m, 0.5H), 5.05 (m, 1H), 5.22 (s, 2H), 7.15-7.45 (m, 4H).

Anal. Calcd. for $C_{20}H_{29}N_2O_6Cl$: C, 56.00; H, 6.82; N, 6.53; Cl, 8.27.

Found: C, 54.55; H, 6.33; N, 6.26; Cl, 8.30.

N^{α}, N^{δ}, N^{ω}-Tribenzyloxycarbonyl-L-arginyl-N^{α}-metyl-N^{ε}-(2-chlorobenzyloxycarbonyl)-L-lysine tert-butyl ester (XVIII-9')

Compound XVIII-7' (4.00 g; 8.9 mmol) was added to a mixture of concentrated sulfuric acid (1.5 mL) in dioxane (35 mL) and stirred for 10 min under an argon purge. Isobutylene (30 mL) was condensed into the reaction mixture using a Dewar reflux condensor. The reaction was stirred for 6 hours, then poured into 1N NaOH (250 mL). The aqueous mixture was extracted using diethyl ether (3×100 mL). The combined ether extracts were washed with saturated $NaHCO_3$ (100 mL) and saturated NaCl (2×100 mL), dried over $MgSO_4$, and evaporated to give 2.05 g of XVIII-8'. N^{α}, N^{δ}, N^{ε}-Tribenzyloxycarbonyl-L-arginine (2.90 g; 5.0 mmol) and isobutylchloroformate (0.78 mL; 6.0 mmol) were added successively. After stirring 20 min, a solution of XVIII-8' in methylene chloride (25 mL) was added to the reaction, followed by N-methylmorpholine (0.53 mL; 5.0 mmol). The reaction was allowed to come to room temperature and stirred for 5 hours. The material was evaporated to an oil and subjected to flash chromatography using a step gradient of 20, 30, and 40% ethyl

acetone in hexane (500 mL each) yielding 2.89 g (30%) of XVIII-9'.

90 MHz ^{1}H-NMR ($CDCl_3$) δ 1.10-2.15 (m, 10H), 1.38 (s, 9H), 2.87 (s, 3H), 3.19 (m, 2H), 3.71 (m, 1H), 3.94 (m, 1H), 4.35 (m, 0.5H), 4.72 (m, 1.5H), 4.95-5.40 (m, 7H), 5.84 (m, 1H), 7.15-7.49 (m, 19H), 9.38 (bs, 2H).

Anal. Calcd. for $C_{49}H_{51}N_6O_{11}Cl$: C, 62.38; H, 6.32; N, 8.91; Cl, 3.76.

Found: C, 62.12; H. 6.11; N, 8.92; Cl, NA.

<u>N^{α}, N^{δ}, N^{ω}-Tribenzyloxycarbonyl-L-arginyl-N^{α}-methyl-N^{ε}-(2-chlorobenzyloxycarbonyl)-L-lysine (XVIII-10')</u>

Compound XVIII-9' (1.42 g; 1.50 mmol) was dissolved in a mixture of trifluoroacetic acid (10 mL) and methylene chloride (10 mL) and stirred for 40 min. The material was concentrated to an oil, diluted in methylene chloride (50 mL), then washed with water (4×50 mL) and saturated NaCl (50 mL), dried over $MgSO_4$, and evaporated to give 1.16 g (87%) of XVIII-10'.

90 MHz ^{1}H-NMR ($CDCl_3$) δ 1.10-2.15 (m, 10H), 2.87 (s, 3H), 3.09 (m, 2H), 3.71 (m, 1H), 3.94 (m, 1H), 4.37 (m, 0.5H), 4.78 (m, 1.5H), 4.95-5.40 (m, 7H), 5.84 (m, 1H), 7.15-7.49 (m, 19H), 9.45 (bs, 3H).

Anal. Calcd. for $C_{45}H_{43}N_6O_{11}Cl$: C, 60.91; H, 5.79; N, 9.47; Cl, 4.00.

Found: C, 60.93; H. 5.84; N, 9.70; Cl, NA.

<u>Solid-phase synthesis of [2-Isopropyl-4-oxo-5(S)-(L-arginyl-N^{α}-methyl-L-lysyl)-amino-6-carboxy-hexanoyl]-</u>

phenylalanine (Isomer A)

The general methodology follows that was previously described in Example 17. Compound XVIII-1 (XI-8 in Example 11) (413 mg, 1.00 mmol) and N-hydroxysuccinimide (121 mg; 1.05 mmol) were dissolved in methylene chloride (20 mL) and cooled to 0°C (ice-bath). DCC (226 mg; 1.10 mmol) was added, and the reaction was stirred at 0°C for 3 hours, then refrigerated overnight. The DCU was filtered off and the filtrate evaporated to give succinimide ester XVIII-2. The Boc group was removed from Merrifield Boc-Phe-O-Resin (2.25 g; 1.50 mmol) by treatment with a cocktail containing 40% TFA and 10% anisole in methylene chloride for 5 min, followed by a second treatement with fresh cocktail for 30 min. The resin was washed with methylene chloride (3x), neutralized with 5% DIEA in methylene chloride (2x) and washed again with methylene chloride (3x). Compound XVIII-2 was dissolved in methylene chloride (10 mL) and added to the resin along with a catalytic amount of HOBt (15 g). The reaction was allowed to proceed for 10 days with periodic monitoring of the supernatant by NMR and yielded compound XVIII-3. The resin was capped by using acetic anhydride (1 mL) and pyridine (0.2 mL) in methylene chloride (10 mL). The Boc group was removed as described above and the resin washed alternately 3 times with methylene chloride and isopropanol, followed by methylene chloride (3x), leaving the resin as the TFA salt.

Meanwhile, dipeptide XVIII-10' (1.06 mg; 1.20 mmol) and HOBt (190 mg; 1.25 mmol) were dissolved in DMF (1 mL), diluted with methylene chloride (10 mL), and cooled to 0°C (ice bath). DCC (270 mg; 1.30 mmol) was added and the resulting mixture stirred at 0°C for 20 min, then at room temperature for 30 min. The resin was neutralized with 5% DIEA in methylene chloride (2x), then washed with methylene chloride (3x), the activated dipeptide was added immediately to the resin. The vessel was shaken overnight. The reaction was judged complete (Kaiser test), and the resin was washed with methylene chloride (2x), isopropanol (2x), and ether (1x), then dried to give 3.35 g of compound XVIII-4. The peptide was cleaved from the resin with concomitant removal of side-chain protecting groups by treating the peptide-resin with 10% anisole in HF (30 mL) at 0°C for 90 min, followed by vacuum distillation of the HF/anisole mixture. The resin was washed with ether, and the peptide extracted with 15% acetonitrile in water containing 0.5% TFA and lyophilized to give 329 mg of crude peptide Arg-NMeLys-Asp(k)Val-Phe ((III) N-MeK^{2}F^{5}-THP). Analytical HPLC indicated that the material consisted of 4 major components. The crude mixture was dissolved in 10% acetonitrile in water containing 0.1% TFA (50 mL) and divided into 5 portions. Each portion was partially purified by preparative HPLC using 10-30% gradients of acetonitrile in water

containing 0.1% TFA. Fractions were combined into 6 pools that contained products corresponding to isomer A of Arg-NMeLys-Asp(k)Val-Phe ((III) N-MeK^{2}F^{5}-THP). A further preparative HPLC purification was performed on each pooled fraction by using an isocratic preparative system of 17% acetonitrile in water with 0.1% TFA as the eluant. This procedure resulted in four fractions of enhanced purity, which were subjected to 12-25% gradients of acetonitrile in water containing 0.1% TFA. Pure fractions were pooled, evaporated of acetonitrile, frozen, and lyophilized to give 23 mg of isomer A of Arg-NMeLys-Asp(k)Val-Phe ((III) N-MeK^{2}F^{5}-THP) (k'=1.76 with 17% acetonitrile in water containing 0.1% TFA). FABS-MS m/e 677 ($M+H^{+}$).

400 MHz ^{1}H-NMR (D_2O) δ 0.86 (d, 6H), 1.37 (m, 2H), 1.62-2.00 (m, 9H), 2.55 (m, 1H), 2.70 (m, 3H), 2.83 (m, 2H), 3.04 (m, 5H), 3.16 (m, 1H), 3.21 (m, 2H), 4.56 (m, 3H), 5.03 (m, 1H), 7.26-7.42 (m, 5H).

Example 19

Preparation of Arg-Nle-Asp-Val(k)Val ((IV) Nle^{2}V^{5}-THP)

6-Methyl-5(S)-tert-butyloxycarbonylamino-4-oxo-2-isopropyl-heptanoic acid attachment to Merrifield chloromethyl resin (XIX-2)

Using an adaptation of the procedure previously described (Arg-Lys-Asp-Val(k)Phe ((IV) F^{5}-THP) in Example 1), the ketomethylene subunit XIX-1 (425 mg; 1.35 mmol)

and cesium bicarbonate (281 mg; 1.45 mmol) were mixed overnight in absolute methanol (25 mL). The mixture was evaporated and the resulting solid was dissolved in DMF (25 mL). Merrifield chloromethyl resin (2.62 g; 2.62 mmol @ 1 meq/g resin) was added and the suspension stirred under argon at 50°C for 48 hours. TLC of the DMF showed no residual ketomethylene XIX-1. The resin was washed numerous times with methylene chloride, methanol, and isopropanol, then dried, to give 2.89 g (ca 0.47 meq/g) of XIX-2.

Solid-phase synthesis of 6-methyl-5(S)-(L-arginyl-L-norleucyl-L-aspartyl)-amino-4-oxo-2-isopropyl-heptanoic acid (Isomer A and Isomer B)

Using the procedure described previously for the synthesis of Arg-Lys-Asp-Val(k)Phe ((IV) F^5-THP) in Example 1, the Boc group was removed from resin XIX-2 (1.43 g; 0.67 meq) through treatment with a cocktail containing 40% TFA and 10% anisole in methylene chloride for five minutes followed by a second treatment with fresh cocktail for 30 min. Following removal of the Boc group, the resin was washed several times alternately with methylene chloride and isopropanol with a final wash with methylene chloride. Meanwhile, dipeptide Boc-Nle-Asp (OBzl)-OH (III-4 in Example 3) (455 mg; 1.0 mmol), DCC (226 mg; 1.1 mmol) and HOBt (153 mg; 1.0 mmol) in DMF (2 mL) were added to methylene chloride (8 mL), stirred at 0°C for 15 min, and then at room temperature for 20

min to prepare the activated ester XIX-3. The activated ester was added to the resin along with diisopropyl-ethylamine (175 µl; 1.0 mmol) and the resulting mixture was shaken overnight. A second coupling was carried out to ensure a complete reaction and formation of XIX-4. The coupling was judged complete (Kaiser test) and the Boc group removed as described above. N^{α}-Boc-N^{ω}-tosyl-L-arginine (574 mg; 1.3 mmol), DCC (304 mg; 1.5 mmol) and HOBt (205 mg; 1.3 mmol) in DMF (2 mL) were added to methylene chloride (8 mL), stirred at 0°C for 15 min, and then at room temperature for 20 min. The resulting activated ester XIX-5 was added to the resin along with diisopropylethylamine (DIEA) (235 µl; 1.3 mmol), and the reaction shaken for 4 hours. A second coupling was performed to ensure complete reaction and formation of XIX-6. The Boc group was removed as described above, the resin was washed and dried; yield 1.75 g. The peptide was cleaved from the resin with concomitant removal of side-chain protecting groups by treating the peptide-resin with 10% anisole in HF (12 mL) at 0°C for one hour followed by vacuum distillation of the HF/anisole mixture. The resin was washed with ether and the peptide extracted with 50% aqueous acetic acid. Lyophilization of the extracts gave 346 mg of crude peptide Arg-Nle-Asp-Val(k)Val ((IV) $Nle^{2}V^{5}$-THP) (86% theor.). The isomers were separated by HPLC using a preparative gradient of 16-30% acetonitrile in water with 0.1% TFA present.

Fractions containing the isomers were pooled, evaporated of acetonitrile, and lyophilized to give 72 mg of isomer A of Arg-Nle-Asp-Val(k)Val ((IV) Nle^2V^5-THP) (99% pure, k'=0.86 with 26% acetonitrile) and 68 mg of isomer B of Arg-Nle-Asp-Val(k)Val ((IV) Nle^2V^5-THP) (99% pure, k'=1.55 with 26% acetonitrile).

Isomer A

FABS-MS m/e 600 ($M+H^+$).

^{1}H-NMR (D_2O) δ 0.77 (d, 3H), 0.83 (m, 3H), 0.86-0.92 (m, 9H), 1.28 (m, 4H), 1.63 (m, 2H), 1.71 (m, 2H), 1.89 (m, 3H), 2.31 (m, 1H), 2.59 (m, 1H), 2.72-2.80 (m, 2H), 2.87 (dd, 1H), 3.00 (dd, 1H), 3.19 (t, 2H), 4.01 (t, 1H), 4.30 (t, 1H), 4.44 (d, 1H).

^{13}C-NMR (D_2O) δ 14.07, 17.19, 19.36, 20.01, 20.39, 22.66, 24.39, 28.12, 29.06, 30.30, 30.63, 31.84, 36.79, 40.81, 41.41, 47.49, 51.28, 53.50, 54.97, 64.50, 157.76, 170.26, 173.15, 174.46, 175.33, 180.51, 211.81.

Anal. Calcd. for $C_{27}H_{49}N_7O_8 \cdot 2CF_3CO_2H$:

C, 44.98; H, 6.21; N, 11.84; F, 13.77.

Found: C, 44.63; H, 6.25; N, 11.75; F, 13.68.

Isomer B

FABS-MS m/e 600 ($M+H^+$).

^{1}H-NMR (D_2O δ 0.82-0.98 (m, 15H), 1.30 (m, 4H), 1.66 (m, 2H), 1.74 (m, 2H), 1.92 (m, 3H), 2.27 (m, 1H), 2.63 (m, 1H), 2.75 (dd, 1H), 2.80 (dd, 1H), 2.90-2.99 (m, 2H), 3.22 (t, 2H), 4.04 (t, 1H), 4.32 (t, 1H), 4.33 (d, 1H).

^{13}C-NMR (D_2O) δ 14.34, 18.08, 20.08, 20.17, 20.60, 22.91, 24.67, 28.40, 29.33, 30.44, 30.88, 32.09, 36.91, 40.32, 41.69, 47.94, 51.49, 53.78, 55.26, 65.67, 158.03, 170.55, 173.45, 174.70, 175.45, 180.68, 212.88.

Anal. Calcd. for $C_{27}H_{49}N_7O_8 \cdot 2.2CF_3CO_2H \cdot H_2O$: C, 43.44; H, 6.17; N, 11.28; F, 14.10.

Found: C, 43.21; H, 5.86; N, 10.91; F, 13.90.

Example 20

Preparation of Arg-Nle-Asp(OMe)-Val(k)Phe(OMe); ((IV) $Nle^2D^3(OMe)F^5(OMe)$-THP)

Esterification of Arg-Nle-Asp-Val(k)Phe; (IV) Nle^2F^5-THP (in Example 3) to give 6-methyl-5(S)-(L-arginyl-L-norleucyl-ß-O-methyl-L-aspartyl)-amino-4-oxo-2-benzyl-heptanoic acid methyl ester (Isomer A)

Compound Arg-Nle-Asp-Val(k)Phe; (IV) Nle^2F^5-THP (in Example 3) (36 mg; 41 µmol) was dissolved in methanol (15 mL). Boron trifluoride etherate (48 µl; 390 µmol) was added and the reaction heated to reflux for one hour. The reaction was cooled, concentrated to an oil, diluted with water, and lyophilized. The product was purified by using a preparative gradient of 10-40% acetonitrile in water with 0.05% TFA present. Fractions containing pure compounds were pooled, evaporated of acetonitrile, and lyophilized to give 25.3 mg (70%) of isomer A of Arg-Nle-Asp(OMe)-Val(k)Phe(OMe) ((IV) $Nle^2D^3(OMe)F^5(OMe)$-THP) (98% pure, k'=1.08, with 36% acetonitrile in water containing 0.1% TFA).

FABS-MS m/e 676 ($M+H^+$).

400 MHz ^{1}H-NMR (CD_3OD) δ 0.80 (d, 3H), 0.91 (t, 3H), 0.92 (d, 3H), 1.39 (m, 4H), 1.68 (m, 2H), 1.77 (m, 2H), 1.92 (m, 2H), 2.23 (m, 1H), 2.62 (dd, 1H), 2.71 (dd, 1H), 2.78 (dd, 1H), 2.86 (dd, 1H), 2.94 (m, 4H), 3.08 (m, 1H), 3.23 (t, 2H), 3.58 (s, 3H), 3.67 (s, 3H), 3.94 (bt, 1H), 4.34 (m, 2H), 4.77 (m, 1H), 7.13-7.28 (m, 5H).

Anal. Calcd. for $C_{33}H_{53}N_7O_8 \cdot 3CF_3CO_2H$: C, 46.01; H, 5.54; N, 9.63.

Found: C, 46.30; H, 5.64; N, 9.27.

Example 21

Preparation of Arg-Lys-Asp(OMe)(k)Val-Phe(OMe); ((III) D^3(OMe)F^5(OMe)-THP)

Esterification of Arg-Lys-Asp(k)Val-Phe; (III) F^5-THP (in Example 11) to give [2-Isopropyl-4-oxo-5(S)-(L-arginyl-L-lysyl)-amino-6-methyloxycarbonyl-hexanoyl]-phenylalanine methyl ester (Isomer A)

Compound Arg-Lys-Asp(k)Val-Phe; (III) F^5-THP (in Example 11) (56 mg; 56 µmol) was dissolved in methanol (20 mL). Boron trifluoride etherate (80 µl; 650 µmol) was added and the reaction heated to reflux for one hour. The reaction was cooled, concentrated to approximately 10 mL, and diluted with water (100 mL); the product was purified by using a preparative gradient of 10-20% acetonitrile in water with 0.05% TFA present. Fractions containing pure compounds were pooled, evaporated of

acetonitrile, and lyophilized to give 28.2 mg (49%) of isomer A of Arg-Lys-Asp(OMe)(k)Val-Phe(OMe) ((III) D^3(OMe)F^5(OMe)-THP) (98% pure, k'=3.17, with 20% acetonitrile in water containing 0.1% TFA).

FABS-MS m/e 691 ($M+H^+$).

^{1}H-NMR (CD_3OD) δ 0.88 (d, 3H), 0.90 (d, 3H), 1.53 (m, 2H), 1.71 (bm, 4H), 1.82 (m, 2H), 1.92 (bm, 1H), 2.02 (bm, 1H), 2.60 (m, 2H), 2.66 (dd, 1H), 2.86 (m, 1H), 2.90 (dd, 1H), 2.95 (t, 2H), 2.99 (dd, 1H), 3.07 (dd, 1H), 3.22 (m, 2H), 3.62 (s, 3H), 3.65 (s, 3H), 4.09 (m, 1H), 4.38 (m, 1H), 4.56 (dd, 1H), 4.61 (dd, 1H), 7.17-7.30 (m, 5H).

Anal. Calcd. for $C_{33}H_{54}N_8O_8 \cdot 3CF_3CO_2H \cdot 2.5H_2O$: C, 43.45; H, 5.80; N, 10.39.

Found: C, 43.34, H, 5.71; N, 10.77.

Example 22

Preparation of Arg(R)Lys-Asp-Val-Phe ((I) F^5-THP (CH_2NH)) N^α, N^δ, N^ω-Tri-benzyloxycarbonyl-L-arginine N-methoxy-N-methyl-amide (XXII-2) Method A

Following the procedure of Fehrenz and Castro (1983), N^α, N^δ, N^ω-tri-benzyloxycarbonyl-L-arginine (2.58 g; 4.48 mmol) was dissolved in methylene chloride and stirred at room temperature. Triethylamine (0.625 mL; 4.5 mmol) and BOP (2.04 g; 4.6 mmol) were successively added to the amino acid solution. After the mixture was stirred for 5 min, N, O-dimethylhydroxylamine hydrochloride (0.498 g; 5.12 mmol) and triethylamine (0.720 g;

5.18 mmol) were added and the resulting reaction was stirred for 2 hours at room temperature. The reaction was diluted with methylene chloride (200 mL) and washed in succession three times with cold 1N HCl (70 mL each), three times with cold saturated sodium bicarbonate (70 mL each), and twice with saturated sodium chloride (70 mL each). The organic layer was dried over magnesium sulfate and evaporated to give the crude product (2.71 g; 98%) as a white solid. The product was subjected to flash chromatography (30mm×200mm bed of EM Science Kieselgel 60, 230-400 mesh ASTM silica gel) eluting with ethyl acetate-hexane (1.0L; 4:6). Pure fractions were pooled and evaporated to yield 2.18 g (79%) of XXII-2.

R_f=0.40 (EtOAc-hexane, 1:1);

^{1}H-NMR ($CDCl_3$) δ 1.65 (m, 4H), 3.12 (s, 3H), 3.63 (s, 3H), 3.93 (m, 2H), 4.70 (m, 1H), 5.07 (s, 2H), 5.12 (s, 2H), 5.21 (s, 2H), 5.47 (d, 1H), 7.43 (s, 15H), 9.35 (bs, 2H).

MS (DCI-NH_3) m/e 620 ($M+H^+$), 486 m/e ($M+H^+-Z$).

Anal. Calcd. for $C_{32}H_{37}N_5O_8$: C, 62.20; H, 6.02; N, 11.30.

Found: C, 62.05, H, 6.01; N, 11.31.

<u>N^α, N^δ, N^ω-Tri-benzyloxycarbonyl-L-arginine N-methoxy-N-methyl-amide (XXII-2) Method B</u>

Following the procedure of Goel et al. (1988), N^α, N^δ, N^ω-tri-benzyloxycarbonyl-L-arginine (11.5 g; 20.0 mmol) was dissolved in a mixture of methylene chloride

and THF (150 mL; 4:1) and the flask was stirred at -15°C for 5 min. N-Methyl morpholine (2.31 mL; 21.0 mmol) and isobutylchloroformate (2.72 mL; 21.0 mmol) were successively added to the reaction vessel and the resulting mixture was stirred at -15°C for 15 min. A pre-cooled solution of N, O-dimethyl hydroxylamine hydrochloride (2.15 g; 22.0 mmol) and N-methylmorpholine (2.42 mL; 22.0 mmol) in methylene chloride (25 mL) was added dropwise to the reaction vessel and the reaction was stirred at -15°C for 5 min, then at room temperature for 4 hours. The reaction was washed in succession three times with ice-cold 0.25N HCl (100 mL each), once with ice-cold water (100 mL), twice with ice-cold saturated sodium bicarbonate (100 mL each), and twice with saturated sodium chloride (100 mL each). The organic layer was dried over magnesium sulfate and evaporated to give 11.8 g (94.8%) of XXII-2 as a white solid, which appeared by TLC to be >98% pure.

^{1}H-NMR ($CDCl_3$): identical to the spectrum described for the product from method A.

<u>N^{α}, N^{δ}, N^{ω}-Tri-benzyloxycarbonyl-L-argininal (XXII-3)</u>

Following an adaptation of the procedure of Goel et al. (1988), lithium aluminum hydride, 95% (0.146 g; 3.66 mmol) was suspended in THF (50 mL) in a three-neck, round-bottomed flask equipped with an overhead stirrer and an addition funnel. The material was stirred rapidly under argon for 30 min, and the resulting fine suspension

was cooled to -50°C (dry ice-isopropanol) and stirred for 15 min. XXII-2 (2.01 g; 3.24 mmol) in THF (10 mL) was added to the reaction dropwise over a 5-min period while keeping the bath temperature at -50°C. Immediately upon the addition of the amino acid, the gelatinous precipitate formed. Hydrogen gas evolution ceased within 2 min of the final addition of the arginine derivative. The cold bath was removed and the reaction was stirred for 5 min with no evidence of further evolution of hydrogen. The reaction was cooled to -50°C again and an aqueous solution (10 mL) of potassium bisulfate (0.890 g; 6.54 mmol) was added carefully over 3 min to the reaction. Hydrogen evolved violently during the addition of the first 2 mL, then ceased. The reaction was stirred at high speed for 20 min to break up the gelatinous product. Ethyl acetate (30 mL) was poured into the reaction vessel and the material was transferred to a separatory funnel. The vessel was rinsed with ethyl acetate (100 mL) and the combined organic solution was washed in succession three times with ice-cold 1N HCl (100 mL each), once with ice-cold water (100 mL), twice with ice-cold saturated sodium bicarbonate (100 mL each), and twice with saturated sodium chloride. The organic layer was dried by passing the solution through a filter pad of magnesium sulfate and was then evaporated to give 1.96 g of XXII-3 as a solid form. The crude material appeared to be >85% pure by TLC. (R_f 0.68 for aldehyde;

0.40 for starting material, 0.15 for minor impurity--in ethyl acetate-hexane (1:1)).

^{1}H-NMR ($CDCl_3$) δ 1.67 (m, 4H), 3.90 (m, 2H), 4.08 (m, 1H), 5.05 (m, 4H), 5.18 (s, 2H), 5.88 (bd, 1H), 7.30 (m, 15H), 9.26 (bs, 2H), 9.45 (s, 1H).

^{1}3C-NMR ($CDCl_3$) δ 24.39, 25.21, 43.95, 59.67, 65.19, 66.92, 68.98, 126.89, 127.54, 127.87, 128.03, 128.36, 128.47, 128.79, 128.90, 134.53, 134.64, 136.21, 136.70, 140.93, 155.66, 155.82, 160.48, 163.57, 199.81.

MS (DCI-NH_3) m/e 561 ($M+H^+$), 427 ($M+H^+$-Cbz).

<u>N^{α}-[2-S-benzyloxycarbonylamino-5-(di-benzyloxycarbonyl)-guanido-pentyl] N^{ε}-benzyloxycarbonyl-L-lysine (XXII-4)</u>

Crude XXII-3 (1.96 g; <3.2 mmol) was dissolved in DMF (10 mL), then diluted with methanol (40 mL). N^{ε}-benzyloxycarbonyl-L-lysine (1.99 g; 7.1 mmol) was added to the flask followed by acetic acid (0.50 mL). An excess of sodium cyanoborohydride (0.632 g; 10.0 mmol) was added in portions over a 3-min period. The reaction was stirred at room temperature under argon overnight. Untreated N-benzyloxycarbonyl-L-lysine was filtered off and the filtrate was evaporated of the methanol. Anhydrous diethyl ether was added to the residual liquid, precipitating a fine white powder. The mixture was stirred 15 min and filtered. The solid material was dissolved in a minimum amount of methylene chloride and reprecipitated with diethyl ether. This afforded the recovery of 1.32 g (50%) of XXII-4.

FABS-MS m/e 825 ($M+H^+$).

90 MHz ^{1}H-NMR ($CDCl_3$) δ 1.05-1.95 (bm, 10H), 2.75-3.25 (bm, 4H), 3.3-4.2 (bm, 5H), 4.9-5.2 (m, 8H), 5.8 (bs, 1H), 7.33 (m, 20H), 9.25 (bs, 2H).

Arg(R)Lys-Asp-Val-Phe ((I) F^5-THP (CH_2NH))

N-Boc-L-Asp(OBzl)-L-Val-Phe-Resin (960 mg of 0.52 meq/g [theoretical]) prepared by standard solid-phase coupling of N^α-Boc-L-Val-OH and N^α-Boc-Asp (OBzl)-OH onto N^α-Boc-L-Phe-O-Merrifield resin; 0.5 mmol) was treated with 40% TFA/10% anisole in methylene chloride for 5 min and then 30 min. The resin was washed with methylene chloride and isopropyl alcohol (3 times each), neutralized with 10% diisopropylethylamine (DIEA), and washed with methylene chloride (3 times). Compound XXII-4 (1.24 g; 1.50 mmol) in methylene chloride (5 mL), HOBt (230 mg; 1.50 mmol) in DMF (2 mL), and DCC (340 mg; 1.65 mmol) in methylene chloride (5 mL) were added successively to the resin reaction vessel and the vessel was shaken overnight. A Kaiser test of the resin indicated that the reaction was complete. The peptide was cleaved from the resin by stirring with 10% anisole in anhydrous HF at 0-5°C for 90 min. After evaporation of HF, the resin was washed with anhydrous diethyl ether (250 mL) and the peptide was extracted with 20% acetonitrile in water containing 0.5% TFA (6×25 mL). The extracts containing peptide were combined and lyophilized to give 340 mg of crude product. The crude material was

purified using 90-min linear preparative gradient of 0-30% acetonitrile in water with 0.1% TFA. Fractions (k'=1.0; with 17% acetonitrile, 100% pure) were pooled and lyophilized to give 212 mg of Arg(R)Lys-Asp-Val-Phe ((I) F^5-THP (CH_2NH)) as a single isomer.

FABS-MS m/e 650 ($M+H^+$).

90 MHz ^{1}H-NMR (CD_3OD) δ 0.91 (dd, 6H), 1.2-2.2 (m, 11H), 2.70-3.05 (m, 5H), 3.05-3.35 (m, 6H), 3.52 (m, 1H), 3.82 (m, 1H), 4.25 (m, 1H), 4.72 (m, 1H), 7.32 (s, 5H).

Anal. Calcd. for $C_{30}H_{51}N_9O_7 \cdot 4CF_3CO_2H$: C, 41.27; H, 5.01; N, 11.40; F, 20.62.

Found: C, 41.08; H, 4.89; N, 11.55; F, 18.68.

Example 23

Solution-Phase Synthesis of Arg-Lys-Asp(k)Val-Phe ((III) F^5-THP)

N-[2-Isopropyl-4-oxo-5(S)-tert-butoxycarbonylamino-6-cyclohexyl-oxycarbonyl-hexanoyl]-L-phenylalanine benzyl ester (Isomer A and Isomer B of XXIII-2)

Phenylalanine benzyl ester tosylate salt (8.55 g; 20.0 mmol) was suspended in methylene chloride (100 mL) and treated with 1N NaOH (100 mL). The layers were separated and the methylene chloride layer washed with saturated NaCl (2×100 mL), dried over $MgSO_4$, and concentrated to approximately 10 mL. Meanwhile, compound XXIII-1 (XI-8 in Example 11) (4.65 g; 11.25 mmol) was dissolved in methylene chloride and cooled to -10°C

(MeOH-ice bath). DCC (2.72 g; 13.2 mmol) and the phenylalanine benzyl ester solution were added successively. The reaction was kept at -10°C for 6 hours, then stirred overnight at room temperature. The reaction was filtered, evaporated to an oil, and subjected to flash chromatography (30x300 mm bed) using a step gradient of 10, 20, 30, and 40% ethyl acetate in hexane (500 mL each). This procedure resulted in the recovery of 0.96 g of the faster eluting diastereomer designated as isomer B of XXIII-2 (upper) (R_f 0.38; 30% EtOAc in hexane), 0.86 g of the slower eluting isomer A of XXIII-2 (lower) (R_f 0.34; 30% EtOAc in hexane), and 2.58 g of a mixture of the two products.

Isomer B of XXIII-2

DCI-MS 651 m/e ($M+H^+$).

90 MHz ^{1}H-NMR ($CDCl_3$) δ 0.82 (d, 6H), 1.0-2.0 (m, 20H), 2.48 (m, 1H), 2.62 (m, 1H), 2.82 (m, 2H), 3.08 (m, 3H), 4.50 (m, 1H), 4.70 (m, 1H), 4.86 (q, 1H), 5.14 (m, 2H), 5.62 (d, 1H), 6.26 (d, 1H), 7.20 (m, 5H), 7.29 (s, 5H).

Anal. Calcd. for $C_{37}H_{50}N_2O_8$: C, 68.28; H, 7.74; N, 4.30.

Found: C, 68.50; H, 7.58; N, 4.15.

Isomer A of XXIII-2

DCI-MS 651 m/e ($M+H^+$).

90 MHz ^{1}H-NMR ($CDCl_3$) δ 0.80 (d, 6H), 1.05-2.00 (m, 20H), 2.45 (m, 1H), 2.6 (m, 1H), 2.78 (m, 2H), 3.08

(m, 3H), 4.50 (m, 1H), 4.80 (m, 2H), 5.12 (m, 2H), 5.71 (d, 1H), 6.32 (d, 1H), 7.20 (m, 5H), 7.35 (s, 5H).

Anal. Calcd. for $C_{37}H_{50}N_2O_8$: C, 68.28; H, 7.74; N, 4.30.

Found: C, 67.86; H, 7.63; N, 4.09.

Solution-phase synthesis of [2-Isopropyl-4-oxo-5(S)-(L-arginyl-L-lysyl)-amino-6-carboxy-hexanoyl]-phenylalanine (Isomer A)

Isomer A of compound XXIII-2 (400 mg; 0.61 mmol) was treated with ethyl acetate (50 mL) that had been saturated with HCl, and the resulting mixture was stirred for 90 min. The material was concentrated to a foam, re-evaporated from ether (3 times), then placed under high vacuum overnight. Dipeptide XXII-5 (XI'-4 in Example 11) (587 mg; 0.70 mmol) and HOBt (115 mg; 0.75 mmol) were dissolved in DMF (1 mL), diluted with methylene chloride (10 mL), and cooled to 0°C (ice-bath). DCC (165 mg; 0.80 mmol) in methylene chloride (2 mL) was added to the reaction, and the solution was stirred at 0°C for 30 min, then at room temperature for 45 min. Isomer A of compound XXIII-4 was dissolved in methylene chloride (10 mL) and added to the activated ester solution. Diisopropylethylamine (110 µl; 0.62 mmol) was added in portions over a 1-hour period, and the reaction was stirred overnight. The material was purified on a silica flash column (30×300 mm) by using a step gradient of methylene chloride (500 mL), 20% ethyl acetate in

methylene chloride (1000 mL), 30% ethyl acetate in methylene chloride (500 mL), and 35% ethyl acetate in methylene chloride (500 mL). Product fractions were pooled and evaporated to give 536 mg (64%) of isomer A of XXIII-7 (>95% pure) and 200 mg (24%) of a mixture of isomer A of XXIII-7 and isomer B of XXIII-7 (isomer ratio 9:1).

Isomer A of XXIII-7

FABS-MS m/e 1370 (M^+).

300 MHz ^{1}H-NMR ($CDCl_3$) δ 0.82 (d, 6H), 1.15-1.55 (bm, 11H), 1.62-1.86 (bm, 10H), 2.42 (m, 1H), 2.56 (m, 1H), 2.73 (m, 2H), 2.97 (m, 1H), 3.04 (m, 4H), 3.92 (m, 2H), 4.29 (m, 2H), 4.68 (m, 2H), 4.87 (m, 1H), 5.06 (m, 8H), 5.18 (m, 1H), 5.21 (s, 2H), 6.05 (d, 0.5H), 6.12 (d, 0.5H), 6.87 (d, 0.5H), 7.08 (m, 1.5H), 7.15-7.38 (m, 31H), 9.32 (bd, 2H).

Anal. Calcd. for $C_{76}H_{90}N_8O_{16}$: C, 66.55; H, 6.61; N, 8.17.

Found: C, 66.41; H, 6.68; N, 8.48.

Isomer A of Arg-Lys-Asp(k)Val-Phe ((III) F^5-THP)

Isomer A of compound XXIII-7 (200 mg; 0.15 mmol) was treated with 10% anisole in HF (5 mL) at 0°C for one hour, followed by vacuum removal of the HF/anisole mixture. The residue was washed with ether, and the peptide was dissolved in water containing 0.5% TFA (100 mL), frozen, and lyophilized. Purification by using preparative HPLC (10-25% gradient of acetonitrile in

water with 0.1% TFA) afforded 107 mg (73%) of isomer A of Arg-Lys-Asp(k)Val-Phe ((III) F^5-THP). The material recovered was identical with material prepared by using the solid-phase technique.

Solution-phase synthesis of [2-Isopropyl-4-oxo-5(S)-(L-arginyl-L-lysyl)-amino-6-carboxy-hexanoyl]-phenylalanine (Isomer B)

Isomer B of compound XXIII-2 (242 mg; 0.37 mmol) was treated with ethyl acetate (50 mL) that had been saturated with HCl, and the resulting mixture was stirred for 30 min. The material was concentrated to a foam, re-evaporated from ether (2 times), then placed under high vacuum overnight. Dipeptide XXIII-5 (XI'-4 in Example 11) (335 mg; 0.40 mmol) and HOBt (63 mg; 0.41 mmol) were dissolved in DMF (0.5 mL), diluted with methylene chloride (10 mL), and cooled to -10°C (methanol-ice bath). DCC (93 mg; 0.45 mmol) was added to the reaction, and the solution was stirred at -10°C for 20 min, then at room temperature for 30 min. Isomer B of compound XXIII-4 was dissolved in methylene chloride (10 mL) and added to the activated ester solution. Diisopropylethylamine (78 µl; 0.45 mmol) was added in portions over a 30-min period, and the reaction was stirred overnight. The material was purified on a silica gel filter pad (60 mL bed volume) by using a step gradient of methylene chloride (200 mL), 25% ethyl acetate in methylene chloride (100 mL), 50% ethyl acetate

in methylene chloride (200 mL). Product fractions were pooled and evaporated to give 320 mg (63%) of isomer B of XXIII-7 (>95% pure).

Isomer B of XXIII-7

FABS-MS m/e 1370 (M^+).

300 MHz ^{1}H-NMR (D_2O) δ 0.72 (d, 6H), 0.76 (d, 3H), 1.09-1.55 (bm, 11H), 1.62-1.86 (bm, 10H), 2.42 (m, 2H), 2.70 (m, 2H), 2.96 (m, 2H), 3.08 (m, 3H), 3.91 (m, 2H), 4.08 (m, 2H), 4.29 (m, 1H), 4.68 (m, 1H), 4.80 (m, 1H), 5.02-5.18 (m, 9H), 5.20 (s, 2H), 6.02 (d, 0.5H), 6.13 (d, 0.5H), 6.91 (d, 0.5H), 7.0 (m, 1H), 7.05 (d, 0.5H), 7.18-7.38 (m, 31H), 9.32 (bd, 2H).

Anal. Calcd. for $C_{76}H_{90}N_8O_{16}$: C, 66.55; H, 6.61; N, 8.17.

Found: C, 66.40; H, 6.68; N, 8.28.

Isomer B of Arg-Lys-Asp(k)Val-Phe ((III) F^5-THP)

Isomer B of compound XXIII-7 (100 mg; 0.07 mmol) was treated with 10% anisole in HF (5 mL) at 0°C for one hour, followed by vacuum removal of the HF/anisole mixture. The residue was washed with ether, and the peptide was dissolved in water containing 0.5% TFA (50 mL), frozen, and lyophilized to give 62 mg (73%) of isomer B of Arg-Lys-Asp(k)Val-Phe ((III) F^5-THP) (96% pure by HPLC). The material recovered was identical with material prepared by the solid-phase technique.

Example 24

Solution-phase synthesis of Arg-NMeLys-Asp(k)Val-Phe ((III) N-MeK^{2}F^{5}-THP; [2-Isopropyl-4-oxo-5(S)-(L-arginyl-N$^{\alpha}$-methyl-L-lysyl)-amino-6-carboxyhexanoyl]-phenylalanine) (Isomer A)

Dipeptide XXIV-3 (XVIII-10' in Example 18) (303 mg; 0.34 mmol) and HOBt (61 mg; 0.40 mmol) were dissolved in DMF (0.5 mL), diluted with methylene chloride (10 mL), and cooled to -10°C (methanol-ice bath). DCC (83 mg; 0.40 mmol) in methylene chloride (5 mL) was added to the reaction, and the solution was stirred at -10°C for 20 min, then at room temperature for 30 min. Compound isomer A of XXIV-2 (isomer A of XXIII-4 in Example 23) (202 mg; 0.34 mmol) was dissolved in methylene chloride (10 mL) and added to the activated ester solution. Diisopropylethylamine (60 µl; 0.34 mmol) was added in one portion, and the reaction was stirred overnight. The material was partially purified on a silica flash pad (60 mL bed volume) by using a step gradient of methylene chloride (150 mL), 20% ethyl acetate in hexane (150 mL), 30% ethyl acetate in hexane (150 mL), 40% ethyl acetate in hexane (150 mL), and 50% ethyl acetate in hexane (150 mL). Product fractions were pooled and evaporated to give 346 mg (71%) of isomer A of XXIV-5 (>90% pure). The material (300 mg; 0.22 mmol) was treated with 10% anisole in HF (5 mL) at 0°C for one hour, followed by vacuum removal of the HF/anisole mixture. The residue was

washed with ether, and the peptide was dissolved in 10% acetonitrile in water containing 1.0% TFA (100 mL), frozen, and lyophilized to give 228 mg of crude isomer A of Arg-NMeLys-Asp(k)Val-Phe ((III) N-MeK^{2}F^{5}-THP). Purification by using preparative HPLC (10-20% gradient of acetonitrile in water with 0.1% TFA) afforded 88 mg (40%) of isomer A of Arg-NMeLys-Asp(k)Val-Phe ((III) N-MeK^{2}F^{5}-THP) (99% pure) and 62 mg of isomer A of Arg-NMeLys-Asp(k)Val-Phe ((III) N-MeK^{2}F^{5}-THP) (90% pure). The material recovered was identical with material recovered from the solid-phase technique.

Isomer A of Arg-NMeLys-Asp(k)Val-Phe ((III) N-MeK^{2}F^{5}-THP)

FABS-MS m/e 677 ($M+H^{+}$).

Anal. Calcd. for $C_{32}H_{52}N_{8}O_{8}\cdot 3CF_{3}CO_{2}H\cdot H_{2}O$: C, 44.01; H, 5.54; N, 10.80.

Found: C, 43.82; H, 5.49; N, 10.55.

Example 25

Competitive Binding Assay

Thymopentin analogs are ranked with respect to overall competitive activity relative to authentic THP in the in vitro CEM cell binding assay described above. The assay is based on the ability of nonradiolabeled THP or nonlabeled analog to compete for binding with tritiated THP. In the absence of a competitor, a fixed amount of bound radioactivity is observed as a function of a set time of exposure and temperature. Nonradiolabeled competitor binding decreases the amount of radioactivity

in proportion to its concentration and relative affinity for the thymopentin receptor.

The analogs are ranked in order of best to worst based on the average radioactivity remaining bound to CEM cells. The concentration of CEM cells was always constant. The data represent the means of determinations made at molar concentrations of added competitor of 10^{-3} and 10^{-4}. The mean total count of radiolabeled THP bound in the absence of competitor was 3078 cpm (N=47); authentic THP reduced the binding to 1150 cpm (N=75). The following results were obtained:

Table 3

Compound	cpm remaining
(III) THP (Isomer A)	653
(III) F^5-THP (Isomer A)	844
(III) F^5-THP (Isomer B)	1091
(III) Nle^2F^5-THP (Isomer A)	1232
(IV) Nle^2F^5-THP (Isomer A)	1254
(III) Nle^2F^5-THP (Isomer B)	1447
(IV) Nle^2F^5-THP (Isomer B)	1453
(IV) F^5-THP (Isomer B)	1673
(IV) $Nle^2A^4F^5$-THP (Isomer A)	1676
(III) P^2F^5-THP (Isomer B)	1686
(I) $Nle^2V^{\dagger 4}F^5$-THP (Isomer B)	1713
(IV) $Nle^2A^4F^5$-THP (Isomer B)	1715
(IV) F^5-THP (Isomer A)	1735
(IV) A^2F^5-THP (Isomer A)	1780

(III)	THP (Isomer B)	1781
(IV)	P^2F^5-THP (Isomer A)	1843
(IV)	L^2F^5-THP (Isomer A)	1851
(I)	F^5-THP (CH_2NH) (Isomer A)	1905
(IV)	P^2F^5-THP (Isomer B)	1999
(IV)	Nle^2V^5-THP (Isomer A)	1600
(IV)	Nle^2V^5-THP (Isomer B)	1713

Example 26

Competitive Binding Assay

The thymopentin analogs of Examples 15, 17, 18, 19 and 22 were tested at three concentrations (10^{-3}M, 10^{-4}M and 10^{-5}M) for their ability to inhibit the binding of a standard amount of ^{3}H-thymopentin (^{3}H-THP).

The following results were obtained:

Table 4

Competition of ^{3}H-THP

Binding to CEM Cells by THP Analogs

Analog	Conc.	^{3}H-THP Binding	% Inhibition
(III) A^4F^5-THP	10^{-3} M	1544	50
(Isomer A)	10^{-4} M	2800	9
	10^{-5} M	3085	-
(III) A^4F^5-THP	10^{-3} M	2219	28
(Isomer B)	10^{-4} M	2994	3
	10^{-5} M	3136	-
(IV) Nle^2V^5-THP	10^{-3} M	1109	64
(Isomer A)	10^{-4} M	2091	32
	10^{-5} M	2926	5

	(IV) Nle^2V^5-THP	10^{-3}	M	1076	65
	(Isomer B)	10^{-4}	M	2351	24
		10^{-5}	M	2826	8
	(IV) N-$MeNle^2F^5$-	10^{-3}	M	1944	37
	THP (Isomer A)	10^{-4}	M	2768	11
		10^{-5}	M	2892	7
	(III) N-Mek^2F^5-THP	10^{-3}	M	2558	17
	(Isomer A)	10^{-4}	M	3082	-
		10^{-5}	M	3656	-
	(I) F^5-THP (CH_2NH)	10^{-3}	M	1477	52
		10^{-4}	M	2524	18
		10^{-5}	M	2462	20
	Authentic THP	10^{-3}	M	586	81
		10^{-4}	M	1512	51
		10^{-5}	M	3018	-

Total binding of ^{3}H-THP was normalized to 3078 cpm.

For comparison to values presented in Table 3, the mean activity of each analog at concentrations of 10^{-3} and 10^{-4} M provide a representative index of competitive binding. The values for each analog and for THP are presented below:

(III) A^4F^5-THP (Isomer A)	2172
(III) A^4F^5-THP (Isomer B)	2606

(IV) Nle^2V^5-THP (Isomer A)	1600
(IV) Nle^2V^5-THP (Isomer B)	1713
(IV) N-MeNle^2F^5-THP (Isomer A)	2356
(III) N-MeK^2F^5-THP (Isomer A)	2820
(I) F^5-THP	2000
Authentic THP	1049*

* This value compares to 1150 in Table 3.

Example 27

Cyclic GMP Assay in CEM Cells

CEM cells were obtained from the American Type Culture Collection (Rockville, MD). The cells were then subcultured in flask at 2×10^5 cells per flask in RPMI-1640 in 20% serum, and stored frozen at 3×10^6 cell/mL in vials for use in the cGMP release assay. The cGMP assay was adjusted to a final concentration of 1×10^7/mL in RPMI medium. After equilibration, the compound to be tested was added for a 2 minute incubation. The reaction was termiated by addition of ice cold TCA. The samples were frozen-thawed three times, and the TCA was removed by ether extraction. After lyophilization, the samples were resuspended in acetate buffer and the cGMP levels were determined by RIA.

The intracellular cyclic GMP levels in CEM cells

in response to thymopentin and thymopentin analogs corroborated the binding assay for analogs (I) Nle^2F^5-THP (Isomer A) and (I) Nle^2F^5-THP (Isomer B) (Table 5). Analog (I) Nle^2F^5-THP (Isomer B) at a concentration of 10^{-3} M induced a two-fold increase in cGMP when compared to control cells ($p=0.08$, Mann-Whitney) whereas addition of analog (I) Nle^2F^5-THP (Isomer A) showed levels comparable to the control cells.

Table 5

Effect of Thymopentin and Thymopentin Analogs on Intracellular cGMP Release in CEM Cells

Sample	N	pmoles cGMP released/ 10^7 cells, Mean ± S.D.
Authentic THP (10^{-3} M)	6	1.8 ± 1.0
(I) Nle^2F^5-THP (Isomer A)	2	3.1 ± 0.3
(I) Nle^2F^5-THP (Isomer B)	3	6.9 ± 3.8

Example 28

Induction of Thy^+-1 Antigen

Induction of the Thy^+-1 antigen in a spleen cell population from nu/nu mice was studied. Quantitation of the induction of the Thy^+-1 antigen was analyzed by fluorescence cell sorting.

Analysis of induction of Thy^+-1 antigen was carried out using spleen cells of nu/nu mice as a source of prethymocytes. The spleen cells were suspended at a concentration of 1×10^7 cells/mL and fractioned using Ficoll/Hypaque separation. The isolated spleen cells

were then adjusted to the final concentration of 1×10^6 cells/mL and exposed to THP or analog (IV) Nle^2F^5-THP (Isomer A) for 4.5 hours at 37°C. The cells were then washed in phosphate buffered saline containing 0.1% azide and treated with FITC-labeled antibody at 4°C for 30 minutes. The cells were then washed by centrifugation through heat-inactivated fetal bovine serum and then fixed for analysis with the fluorescence activated cell sorter.

Both THP and (IV) Nle^2F^5-THP (Isomer A) at concentrations of 10^{-3} M increased the proportion of cells showing Thy^+-1 antigen. In the presence of either THP or (IV) Nle^2F^5-THP (Isomer A), the Thy-1 positive cells were increased from 25% in untreated controls to 38% and 46%, respectively. Representative profiles obtained for the cellular distribution of the Thy^+-1 positive cells following treatment THP and analog (IV) Nle^2F^5-THP (Isomer A) were shown in Table 6.

Table 6

Induction of Thy-1 Antigen

Sample	Thy-1 Positive Cells (%)
Untreated	25 %
Authentic THP (10^{-3} M)	38 %
(IV) Nle^2F^5-THP (Isomer A)	46 %

Example 29

Determination of Serum Stability of Thymopentin Analogs by HPLC Analysis

Analysis of serum half-lives of THP analogs was carried out by incubation of the test compound with heparinized human blood plasma and EDTA-treated mouse blood plasma at 37°C for variable, specified time periods ranging from 0 to 10 minutes. The serum incubation was terminated by addition of trifluoroacetic acid (TFA) to a final concentration of 10% v/v. The mixture was maintained at 0°C for 1 hour and the proteins were removed by centrifugation (10,000 × G for 15 minutes). The supernatants were collected and stored at -20°C until HPLC analysis.

Elutions were carried out using isocratic gradients of acetonitrile in water in the presence of TFA. Identification of THP, THP analogs and degradation products was performed using peak elution times as an index of time-dependent conversion between starting material and metabolite.

Results showed that compounds that gave good results in the THP competitive binding assay also exhibited the maximum extension of serum half-life. These results are shown in Table 7.

Table 7

Compound	Half-Life (min)	
	Mouse	Human
Authentic THP	0.5	1.5
Arg-Lys-Asp-Val(k)Phe (Isomer A)	4.9	2.0
Arg-Pro-Asp-Val(k)Phe (Isomer A)	17.0	6.0

Arg-Nle-Asp-Val(k)Phe (Isomer A)	21.1	1.9
N-Ac-Arg-Nle-Asp-Val(k)Phe (Isomer A)	>32.0	5.1
Arg-Lys-Asp-Val(k)Phe (Isomer A)	5.8	2.7
Arg(k)Nle-Asp-Val-Phe	-	12.5
Arg-Nle-Asp-Val(k)Phe (Isomer A)	20.0	2.5
Arg-NMeNle-Asp-Val(k)Phe (Isomer A)	>32.0	>32.0
Arg-NIe-Asp-Val(k)Val (Isomer A)	1.2	2.4
Arg-Nle-Asp-Val(k)Val (Isomer B)	2.6	2.4
Arg-NMeNle-Asp-Val(k)Val (Isomer A)	>32.0	>32.0
Arg-NMeNle-Asp-Val(k)Val (Isomer B)	>32.0	>32.0
Arg-NMeLys-Asp(k)Val-Phe (Isomer A)	>32.0	>32.0
Arg(R)Lys-Asp-Val-Phe	>32.0	13.8

CLAIMS

1. A pseudopeptide of the formula

I II III IV

$$R\text{-}AA_1\text{--}AA_2\text{--}AA_3\text{--}AA_4\text{--}AA_5\text{-}R_1$$

wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a basic amino acid residue, a neutral/nonaromatic amino acid residue or proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid or glutamic acid residue, wherein the remaining carboxyl group may optionally be esterified with alkyl (1-6C), or alanine residue;

AA_4 is an L- or D-form of a neutral/nonaromatic amino acid residue;

AA_5 is an L- or D-form of a neutral/aromatic amino acid residue wherein one or more hydrogens of its aromatic portion can be substituted by NO_2 or halogen or is an L- or D-form of a neutral/nonpolar/large/non-aromatic amino acid residue or the N-alkylated (1-6C) form of the above;

R is acyl (1-6C), arylsulfonyl, alkylsulfonyl, arylalkylsulfonyl or alkoxycarbonyl group;

R_1 is -OH, $-NR_2R_3$ or $-OR_4$ wherein each of R_2 and R_3 is independently hydrogen or an alkyl group (1-6C) and R_4 is alkyl group (1-6C);

wherein at least one of the linkages numbered I-IV is a modified peptide linkage selected from the group consisting of $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2NH-$, and the remaining linkages are $-CONH-$ or $-CON(CH_3)-$,

and the pharmaceutically acceptable salts thereof.

2. A pseudopeptide of the formula

I II III IV

$$R-RR_1--AA_2--AA_3--AA_4--AA_5-R_1$$

wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a basic amino acid residue, a neutral/nonaromatic amino acid residue or proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid residue wherein the remaining carboxyl group may optionally be esterified with alkyl (1-6C);

AA_4 is an L- or D-form of a neutral/nonaromatic amino acid residue;

AA_5 is an L- or D-form of a neutral/aromatic amino acid residue wherein one or more hydrogens of its aromatic portion can be substituted by NO_2 or halogen or is an L- or D-form of a valine residue, or is an N-alkylated (1-6C) form of the above;

R is acyl (1-6C), arylsulfonyl, alkylsulfonyl, arylalkylsulfonyl or alkoxycarbonyl group;

R_1 is $-OH$, $-NR_2R_3$ or $-OR_4$ wherein each of R_2 and R_3

is independently hydrogen or an alkyl group (1-6C) and R_4 is alkyl group (1-6C);

wherein at least one of the linkages numbered I-IV is a modified peptide linkage selected from the group consisting of $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2NH-$, and the remaining linkages are $-CONH-$ or $-CON(CH_3)-$,

and the pharmaceutically acceptable salts thereof.

3. A pseudopeptide of the formula

I II III IV

$$R-AA_1--AA_2--AA_3--AA_4--AA_5-R_1$$

wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a basic amino acid residue, a neutral/nonaromatic amino acid residue or proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid residue wherein the remaining carboxyl group may optionally be esterified with alkyl (1-6C);

AA_4 is an L- or D-form of an alanine or valine residue;

AA_5 is an L- or D-form of a neutral/aromatic amino acid residue wherein one or more hydrogens of its aromatic portion can be substituted by NO_2 or halogen or is an L- or D-form of a valine residue, or is an N-alkylated (1-6C) form of the above;

R is acyl (1-6C), arylsulfonyl, alkylsulfonyl,

arylalkylsulfonyl or alkoxycarbonyl group;

R_1 is -OH, $-NR_2R_3$ or $-OR_4$ wherein each of R_2 and R_3 is independently hydrogen or an alkyl group (1-6C) and R_4 is alkyl group (1-6C);

wherein at least one of the linkages numbered I-IV is a modified peptide linkage selected from the group consisting of $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2NH-$, and the remaining linkages are -CONH- or $-CON(CH_3)-$,

and the pharmaceutically acceptable salts thereof.

4. A pseudopeptide of the formula

I II III IV

$$R-AA_1--AA_2--AA_3--AA_4--AA_5-R_1$$

wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a basic amino acid residue, a neutral/nonaromatic amino acid residue or proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid residue wherein the remaining carboxyl group may optionally be esterified with alkyl (1-6C);

AA_4 is an L- or D-form of an alanine or valine residue;

AA_5 is an L- or D-form of a phenylalanine or tyrosine residue wherein one or more hydrogens of its aromatic portion can be substituted by NO_2 or halogen or is an L- or D-form of a valine residue, or is an

N-alkylated (1-6C) form of the above;

R is acyl (1-6C), arylsulfonyl, alkylsulfonyl, arylalkylsulfonyl or alkoxycarbonyl group;

R_1 is -OH, $-NR_2R_3$ or $-OR_4$ wherein each of R_2 and R_3 is independently hydrogen or an alkyl group (1-6C) and R_4 is alkyl group (1-6C);

wherein at least one of the linkages numbered I-IV is a modified peptide linkage selected from the group consisting of $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2NH-$, and the remaining linkages are -CONH- or $-CON(CH_3)-$,

and the pharmaceutically acceptables salts thereof.

5. A pseudopeptide of the formula

I II III IV

$$R-AA_1--AA_2--AA_3--AA_4--AA_5-R_1$$

wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a lysine, alanine, α-aminoisobutyric acid, leucine, norleucine or proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid residue wherein the remaining carboxyl group may optionally be esterified with alkyl (1-6C);

AA_4 is an L- or D-form of an alanine or valine residue;

AA_5 is an L- or D-form of a phenylalanine or tyrosine residue wherein one or more hydrogens of its aromatic portion can be substituted by NO_2 or halogen or

is an L- or D-form of a valine residue, or is an N-alkylated (1-6C) form of the above;

R is acyl (1-6C), arylsulfonyl, alkylsulfonyl, arylalkylsulfonyl or alkoxycarbonyl group;

R_1 is -OH, $-NR_2R_3$ or $-OR_4$ wherein each of R_2 and R_3 is independently hydrogen or an alkyl group (1-6C) and R_4 is alkyl group (1-6C);

wherein at least one of the linkages numbered I-IV is a modified peptide linkage selected from the group consisting of $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2NH-$, and the remaining linkages are -CONH- or $-CON(CH_3)-$,

and the pharmaceutically acceptable salts thereof.

6. The pseudopeptide of any one of claims 1-5 wherein said substitution of a modified linkage is at only one of the linkages I-IV.

7. The pseudopeptide of any one of claims 1-5 wherein R is acetyl group.

8. The pseudopeptide of any one of claims 1-5 wherein R_1 is NH_2 or OH.

9. The pseudopeptide of any one of claims 1-5 which is selected from the group consisting of

Arg-Lys-Asp-Val(k)Phe;

Arg-Pro-Asp-Val(k)Phe;

Arg-Nle-Asp-Val(k)Phe;

Arg-Lys-Asp-Ala(k)Phe;

N-Ac-Arg-Lys-Asp-Val(k)Phe;

Arg-Lys-Asp-Val($CHOHCH_2$)Phe;

Arg-Nle-Asp-Ala(k)Phe;

Arg-Nle-Asp-Val($CHOHCH_2$)Phe;

Arg-NMeNle-Asp-Val(k)Phe;

Arg-Leu-Asp-Val(k)Phe;

N-Ac-Arg-Pro-Asp-Val(k)Phe;

N-Ac-Arg-Nle-Asp-Val(k)Phe;

Arg-D-Lys-Asp-Val(k)Phe;

Arg-Nle-Asp-Val(k)Val;

Arg-NMeLeu-Asp-Val(k)Phe;

Arg-Ala-Asp-Val(k)Phe;

Arg(k)Lys-Asp-Val-Phe;

Arg(k)Nle-Asp-Val-Phe;

Arg(k)Nle-Asp-Val-Phe-$CONH_2$

Arg(k)Nle-Asp-D-Val-Phe;

Arg-Lys-Asp(k)Val-Phe;

Arg-Nle-Asp(k)Val-Phe;

Arg-Pro-Asp(k)Val-Phe;

N-Ac-Arg-Lys-Asp(k)Val-Phe;

Arg-Pro-Asp(k)Val-Phe-$CONH_2$;

Arg-Lys-Asp(k)Ala-Phe;

Arg-Lys-Asp(k)Val-Tyr;

Arg-NMeLys-Asp(k)Val-Phe;

Arg-Nle-Asp(k)Val-Tyr;

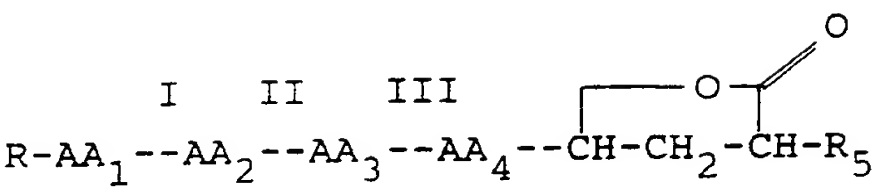
Arg(CH_2NH)Lys-Asp-Val-Phe;

Arg-Nle-Asp(OMe)-Val(k)Phe(OMe);

Arg-NMeNle-Asp-Val(k)Val; and

Arg-Lys-Asp(OMe)(k)Val-Phe(OMe).

10. A pseudopeptide of the formula



wherein AA_1 is an L- or D-form of an arginyl residue;

AA_2 is an L- or D-form of a lysine, alanine, α-aminoisobutyric acid, leucine, norleucine or proline residue, or is an N-alkylated (1-6C) form thereof;

AA_3 is an L- or D-form of an aspartic acid residue wherein the remaining carboxyl group may optionally be esterified with alkyl (1-6C);

AA_4 is an L- or D-form of an alanine or valine residue;

R is an acyl group (1-6C), arylalkylsulfonyl group, alkylsulfonyl group, arylsulfonyl group, or alkoxy-carbonyl group;

R_5 is selected from the group consisting of a benzyl optionally substituted by NO_2 or halogen, 4-hydroxy-benzyl optionally substituted by NO_2 or halogen, and isopropyl group, or is an N-alkylated (1-6C) form thereof;

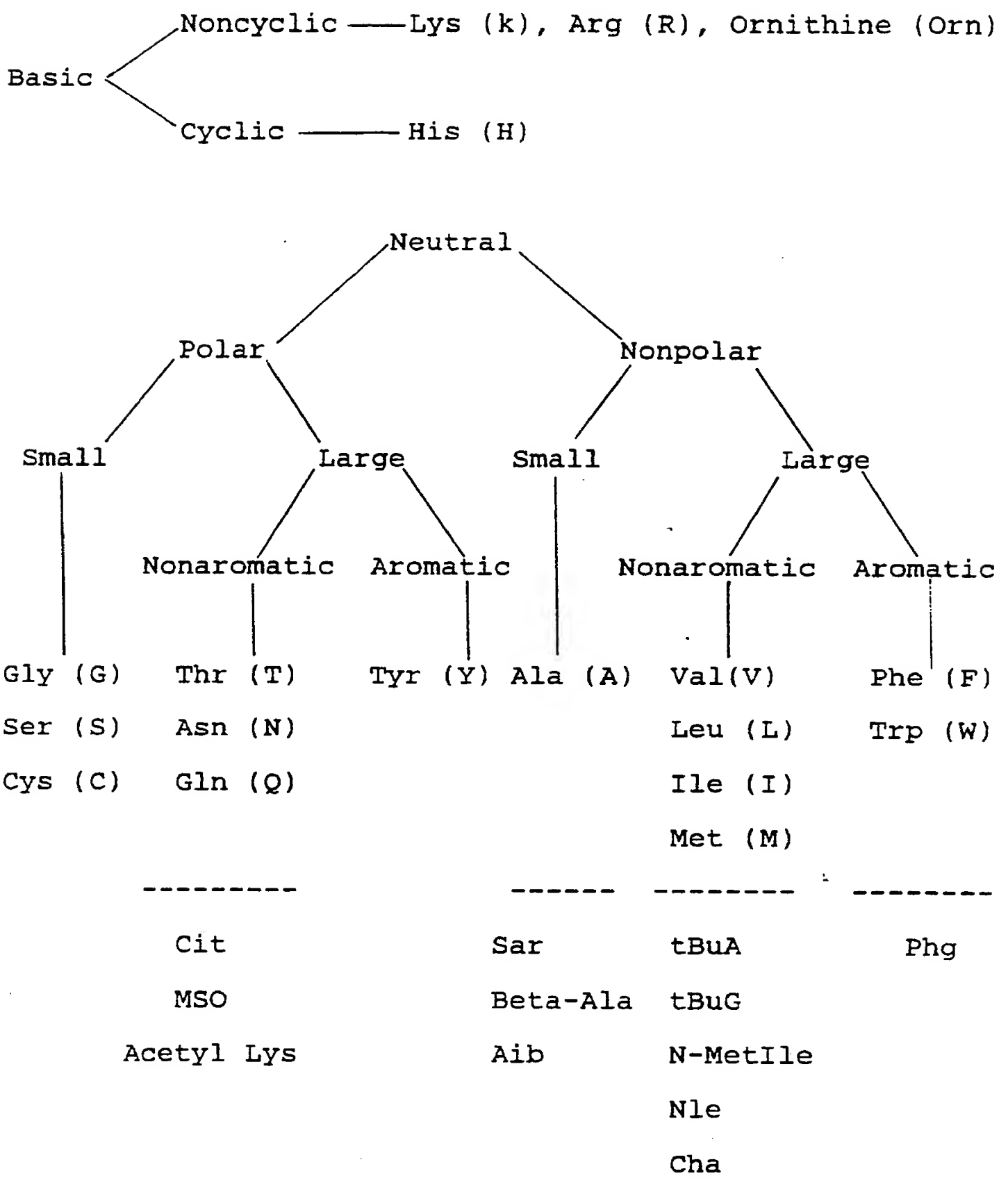
wherein linkages numbered I-III are each independently -CONH- or $-CON(CH_3)-$;

and the pharmaceutically acceptable salts thereof.

11. A pharmaceutical composition, which comprises an effective amount of the pseuopeptide of any of claims 1-5

or 10 in admixture with a pharmaceutically acceptable excipient.

Fig. 1



INTERNATIONAL SEARCH REPORT

International Application No PCT/JP 92/01046

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]
According to International Patent Classification (IPC) or to both National Classification and IPC
Int.Cl. 5 C07K5/02; C07K7/02; A61K37/02; C07K15/00

II. FIELDS SEARCHED	
Minimum Documentation Searched[7]	
Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]	

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	EP,A,0 393 786 (SCLAVO) 24 October 1990 see the whole document ---	1-11
A	EP,A,0 342 962 (IMMUNOBIOLOGY RESEARCH INSTITUTE) 23 November 1989 see the whole document -----	1-11

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 NOVEMBER 1992	[illegible]
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P. masturzo

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. JP 9201046 SA 63691

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 17/11/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0393786	24-10-90	CA-A- 2015053	21-10-90
		JP-A- 2292250	03-12-90
EP-A-0342962	23-11-89	AU-B- 625598	16-07-92
		AU-A- 3745589	12-12-89
		CN-A- 1037711	06-12-89
		JP-T- 3504013	05-09-91
		WO-A- 8911289	30-11-89

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82